DISCOVERY AND MECHANISTIC CHARACTERIZATION OF POTENTIAL PLANT-DERIVED MALARIA TRANSMISSION-BLOCKING AGENTS

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Discovery and Mechanistic Characterization of Potential Plant-Derived Malaria Transmission-Blocking Agents

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other university.

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DEDICATION

This PhD thesis is whole-heartedly dedicated to;

My beloved family, the Muema's, my wife Mary, our daughter Stephanie Zawadi, and in loving memory of my late grandfather who during his life inspired my today's hidden treasure of medicinal plants

Science is the differential calculus of the mind. Art the integral calculus; they may be beautiful when apart, but are greatest only combined.
~Ronald Ross 1857-1932

Above all, never be afraid to take the path less travelled….
Sometimes life's greatest lessons are learned outside our comfort zone… to make that difference!
~ Robert Frost 1874–1963

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LIST OF ABBREVIATIONS

ACT	Artemisinin Combined Therapy
AP2-G	*Plasmodium* Apetala 2-G transcription factor
cDNA	Complementary DNA
CH_2Cl_2	Dichloromethane
CITH	CAR-I in fly and Trailer Hitch
CQ	Chloroquine
CSP	Circumsporozoite Protein
CTRP	Circumsporozoite Thrombospondin Related anonymous Protein
DHA	Dihydroartemisinin
DOZI	Development of Zygote Inhibited
dsRNA	Double stranded ribonucleic acid
ECG	Epicatechin gallate
ED_{50}	Effective dose at 50%
EGCG	Epigallocatechin gallate
EIR	Entomological Inoculation Rate
G6PD	Glucose-6-Phosphate Dehydrogenase
GMEP	Global Malaria Eradication Programme
GSK	GlaxoSmithKline
IACUC	Institutional Animal Care and Use Committee
IC_{50}	Inhibitory Concentration that suppresses growth of 50% of the cell population
icipe	International Centre of Insect Physiology and Ecology

IRS	Indoor Residual Spraying
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
LLINs	Long Lasting Insecticide-treated Nets
LRIM1	Leucine-rich Repeat Immune Protein 1
m	Meters
M	Molar
m/z	Mass to charge ratio
MAPK	Mitogen Activated Protein Kinase
MeOH	Methanol
mg/kg/day	Milligrams per kilogram body weight per day
MMV	Medicines for Malaria Venture
nM	Nanomolar
NOS	Nitric Oxide Synthase
PCR	Polymerase Chain Reaction
ppm	Parts Per Million
QN	Quinine
***q*RT-PCR**	Quantitative Reverse Transcription Polymerase Chain Reaction
RNA*i*	RNA interference
SI	Selectivity Index
SOAP	Secreted Ookinete Adhesive Protein
TBVs	Transmission-Blocking Vaccines
TEP1	Thioester-containing Protein 1

TRAP	Thrombospondin Related Anonymous Protein
µg/mL	Micrograms per millilitre
µM	Micromolar
USAMRD-A/K	US Army Medical Research Directorate-Africa, Kenya
WARP	von Willebrand factor A domain-related protein
WHO	World Health Organization

ABSTRACT

The renewed global focus on malaria elimination and eradication through successful reduction of *Plasmodium* transmissions by infected mosquitoes warrants the discovery of effective anti-infectives targeting the transmissible parasite stages and their vectors. Owing to the toxicity of primaquine, the only approved drug that is effective against the stage V gametocytes, safer therapeutics are urgently needed to combat transmissions. Recognition of the important role of plants in antimalarial drug discovery was first through the isolation of quinine and artemisinin. However, few plant-derived agents inhibit gametocytes development or sexual gametocytogenesis pathway to facilitate transmission-blocking of malaria parasites, underscoring their unmet demand. In addition, their underlying molecular mechanisms of action remain uncharacterized. This necessitates discovery of new plant-derived malaria transmission-blocking agents targeting the *Plasmodium* gametocytes and mosquito larvae. In the quest to expand the malaria transmission-blocking chemical space and elucidate molecular targets, this study evaluated extracts from 13 selected plants. The goal was to mine for potent malaria control agents with novel molecular mechanisms of action. In addition, the impact of most bioactive compound on mosquito vector fitness, and characterization of larvicidal effects of *Zanthoxylum chalybeum* root constituents on mosquito larvae growth were assessed. From the 13 plant extracts tested against *Plasmodium falciparum* multidrug-resistant asexual parasites (PfW2 strain), two active antimalarial extracts from *Prosopis juliflora* (IC_{50} 1.02 µg/mL) and *Cissampelos pariera* (IC_{50} 2.09 µg/mL) also exhibited potent *Plasmodium* late-stage IV/V gametocytes from NF54 and human clinical isolates within the submicromolar IC_{50} window. Based on LC-ESI-MS/MS analyses, the observed antimalarial activity was found to be exerted by a known 2,3-dihydro-*1H*-indolizidinium, juliprosopine from *P. juliflora* (IC_{50} 0.604 µg/mL for D6 *Plasmodium* isolate), and previously isolated bisbenzylisoquinoline (BBIQ), isoliensinine from *C. pariera* (IC_{50} 1.329 µg/mL for D6), respectively. The two antimalarial compounds inhibited *P. falciparum* Dd2 trophozoites-to-schizont transformation of the 48-h intraerythrocytic cycle suggesting impairment of cell cycle regulatory elements. Further prioritized activity-profiling of juliprosopine showed potent blockade of *Plasmodium* gametocytogenesis of NF54 strain by day 7 post-induction, without exerting deleterious effects on female *Anopheles* mosquito survival and egg hatchability. Insights into the possible molecular targets predicted similar parasite biological pathways predominantly clustering into: protein modifications, cell cycle and chromatin remodeling, fatty acid biosynthesis, and host cell protein export, described during late trophozoites and late-stage IV/V gametocytes. Evaluation of *Zanthoxylum chalybeum* Engl (ZCE)(Rutaceae) root constituents against mosquito larvae demonstrated dose-dependent biphasic effects on larval treatment; (i) transient exposure to ZCE and its bioactive fraction (ZCFr.5) inhibited acetylcholinesterase (AChE) activity thus inducing larval lethality and (ii) growth retardation at sublethal doses. The half-maximal lethal concentrations (LC_{50}) for the mosquito larvae ranged between 1.58 – 12.26 ppm, exerted by 2-tridecanone, palmitic acid (hexadecanoic acid), linoleic acid ((Z,Z)-9,12-octadecadienoic acid), sesamin, β-caryophyllene, among other compounds identified in bioactive ZCFr.5

fraction. The observed larval growth retardation induced by ZCE root constituents were exerted through intracellular transcriptional modulation of ecdysteroidogenic CYP450 genes. Collectively, the key findings of this study necessitate further explorative optimizations of the identified molecules for the development of potential malaria control interventions and functional mechanism validations.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Prior to the remarkable 1880 and 1897 discoveries, cyclical malarial fever was mystically presumed to be an influence of "bad air" or miasma emanating from low-lying swamps and marshes (Hempelmann & Krafts, 2013). The disease now called malaria, is aetiologically caused by hemacytozoon *Plasmodium* parasites. These parasites were discovered as pathological pigments by French Army Surgeon Major Charles Louis Alphonse Lavern in Algerian infected blood samples, and further as peculiar infectious organisms in female *Anopheles* mosquito midgut by British Officer Sir Ronald Ross (Ross, 1897; Cox, 2010; Hempelmann & Krafts, 2013). Many years since then, attention has been channeled to the pathology-causing intraerythrocytic asexual blood stages and apparently neglected the transmissible sexual stages that sustain epidemiological infections. Despite these serendipitous contributions to the basic understanding of malaria origin and incrimination of mosquitoes for its spread, a lot has remained less understood regarding the sexual differentiation, transmission, and effective control of *Plasmodium* stages infective to the vector. Until recent past, information on sexual stages was quite scanty (Alano, 2007). However, with the advent of 'omics' technologies during the 21st Century, renewed efforts from the international scientific community geared towards malaria elimination have facilitated progressive study of *Plasmodium* transmission biology (Alonso et al., 2011; Sinden et al., 2012). This has been exemplified by sequencing of *P. falciparum* and *An. gambiae* genomes in 2002 that continues to provide insights into vector-parasite interactions as well as reveal new avenues for discovery of transmission-blocking interventions.

With-intention-to-kill *Plasmodium* parasites before they are transmitted to mosquitoes, transmission-blocking strategies are proposed and several attempts to develop biomolecules targeting the parasites Achilles heel are currently underway (Bompard *et al*., 2017; Burrows *et al*., 2017). Currently, no clinically approved vector-based transmission blocking vaccine (TBV) reported, leaving chemotherapy the only option to venture. Several open chemical libraries such as the medicines for malaria venture (MMV) malaria box have been developed to accelerate search of novel antimalarials. However, a myriad of these compounds is expected to fail during clinical trials despite showing promising antimalarial potential due to high attrition rates. Consequently, few compounds exhibit either selective inhibition of the late-stage V gametocytes or dual inhibition of asexual and sexual blood stages, and discovery of such new chemistries is desperately warranted to meet the increasing demand of antimalarials (Sinden, 2017). In search for safer prophylactic compounds, alternative natural sources are currently pursued.

Naturally-occurring compounds are targeted source of novel pharmacophores for treatment and transmission-blocking of malaria (Li & Vederas, 2009; Gisburg & Deharo, 2011; Guantai & Chibale, 2011; Wells, 2011; Onguéné et al., 2013; Ntie-Kang et al., 2014). The implication of plant-derived compounds in providing prophylactic effects is well known, and to-date only few compounds have reported to exhibit *Plasmodium* transmission-blocking activities. These include Azadirachtin A from *Azadirachta indica*, vernodalol from *Vernonia amygdalina*, parthenin and parthenolide from *Parthenium hysterophorous* and *Tanacetum parthenium* respectively, and daucovirgolide G from *Daucus virgatus*. None of these compounds has well-defined molecular targets. The current study aimed at screening for more potential malaria transmission-blocking agents from selected tropical Kenyan plants and elucidating their possible mechanisms of action.

1.2 Statement of the problem

The late-stage V *Plasmodium* gametocytes sustainably maintain human-to-mosquito transmission and malaria burden experienced in Africa and other resource-constrained developing countries of the world despite great efforts of control. Although the current artemisinin and its partner combinatorial agents (ACTs) have progressively reduced malaria infections, they are ineffective against these gametocytes thus promoting residual infectivity to mosquitoes from the treated individuals. The malaria control is further thwarted by the increasing reports of antimalarial drug resistance, widespread physiological and behavioral insecticide resistance, absence of a viable transmission-blocking vaccine and an existing knowledge gap between *Plasmodium* transmission, mosquito ecology, and intervention deployment.

In the wake of global efforts to eliminate malaria, effective transmission-blocking interventions could significantly contribute to malaria reductions if available. Toxicity limitations by the only approved gametocytocidal drug molecule, primaquine, and restricted efficacy of artemisinin against only immature gametocytes calls for concerted efforts towards discovery of new antimalarial agents, in addition to vector control. Towards this end, and with a desperate need for new arsenals to break the human-mosquito-human *Plasmodium* transmission cycle, renewed efforts have turned to nature in search of such chemical agents possessing new mechanisms of action. Through the use of indigenous knowledge, plants can continue to provide potent anti-infective pharmacophores, in addition to prophylactic herbal remedies for humans. To-date, only five (5) plant-derived agents have been reported to display inhibitory activities against *Plasmodium* transmissible stages or potentially blocked transmission to *Anopheles* mosquitoes. Taken together, discovery of more novel and safer transmission-blocking agents is imperative to reduce malaria burden and promote local disease elimination.

1.3 Justification of the study

Interventions to break plasmodial transmission cycle are required to reduce disease burden and improve human wellbeing. Among these are transmission-blocking agents. There is increasing demand for discovery of new and effective antimalarials particularly targeting the transmissible stages. Naturally occurring compounds provide alternative sources of effective scaffolds for development of new chemotherapeutics against various parasitic diseases. With only few plant-derived compounds hitherto reported to exhibit gametocytocidal and inhibitory activities against sporogonic stages, more are required to meet the demand. Furthermore, none of these compounds has been functionally characterized to elucidate their mode of action. To discover new potent antimalarials and expand the existing chemical library, 13 Kenyan plants were selected based on their chemotaxonomic profiles and previously reported bioactivities for transmission-blocking evaluations. The identified pharmacophores scaffolds, and their functional analyses can open up proteomics avenues towards design and optimization of potential transmission-blocking antimalarials for individuals residing within endemic settings, travellers, and military deployed in these affected regions.

1.4 Research questions

(i) Could extracts of the selected plants sufficiently kill *Plasmodium falciparum* gametocytes development?

(ii) What bioactive constituents of the potent plant extracts were eliciting the gametocytocidal activities and their possible targets?

(iii) Do the potent plant-derived antimalarial compounds elicit any deleterious effect on mosquito vector fitness?

(iv) Could *Zanthoxylum chalybeum* be explored for potential mosquito larvicides with novel molecular target mechanism?

1.5 Objectives of the study

1.5.1 General objective

To discover potential malaria transmission-blocking compounds from selected terrestrial Kenyan plants and functionally elucidate their possible molecular mechanisms of action.

1.5.2 Specific objectives

(i) To identify potent *Plasmodium falciparum* gametocytocidal extracts from selected terrestrial plants

(ii) To determine the bioactive chemical entities mediating gametocytocidal activity in *Cissampelos pareira* and *Prosopis juliflora* and postulate their molecular targets

(iii) To explore the effects of potent juliprosopine isolated from *P. juliflora* on female *Anopheles* mosquito fitness

(iv) To characterize the larvicidal activity of *Z. chalybeum* root constituents against mosquito larvae and determine their molecular effects.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria transmission and regulation: sowing cunning seeds of death

2.1.1 Commitment to gametocytogenesis under transcriptional switches

Transmission of *Plasmodium* parasites to the mosquito vector from infected human hosts is hallmarked by cellular differentiation of sexually committed merozoites into morphologically distinct gametocytes through stringently-regulated processes (Figure 2.1). Under natural host environment, a small number of gametocytes (~ 0.1% to 5%) develop from a heterogenous pool of asexual and sexual blood stages, characterizing the first population bottleneck and a viable target for transmission blocking (Nilsson et al., 2015). Commitment to leave asexual replication into sexual transitions has been reported to occur at schizont stage before the release of sexually pre-determined merozoites (Bruce et al., 1994). Different *Plasmodium* species exhibit varied time lengths of gametocyte maturation with *P. falciparum* taking ~10 - 12 days, whereas the rodent *P. berghei* lasts for 28 days while maintaining a spherical shape during growth (Waters & Janse, 2004). Notwithstanding, the precise timing, mechanistic triggers, and functional molecular signatures of gametocytogenesis remain yet-to-be fully unraveled though progressively being illuminated (Baker, 2010; Meibalan & Marti, 2017). Although gametocytogenesis is more complex under natural settings and induced by extraneous factors, *in vitro* model studies have reported the involvement of exogenous factors such as; high asexual parasite density, host-derived lysophosphatidylcholine (lysoPC), presence of extracellular microvesicles, host red blood cells (RBCs) age, host hormones, antimalarial drug exposure, homocysteine, and endoplasmic reticulum stress (Mantel et al., 2013; Chaubey et al., 2014; Beri et al., 2017; Brancucci et al., 2017; Zuzarte-Luís & Mota, 2018).

At the molecular level, initial investigations associated a set of genes located on chromosome 9 with *Plasmodium* schizont commitment (Day et al., 1993; Alano et al., 1995). *P. falciparum* gene implicated in gametocytogenesis (*Pfgig*; *Pf*3D7_

0935600/*Pf*I1720w) and gametocyte development gene 1 (*Pf*gdv1; Pf3D7_ 0935400/PFI1710w) were established, through targeted gene deletion studies, to functionally regulate gametocytogenesis (Gardiner et al., 2005; Eksi et al., 2012). On disruption these genes, it was noted that development of gametocytes reduced and expression of known gametocyte-specific genes such as *Pfs16* (Pf3D7_ 0406200/PFD0310w) was only induced upon gene complementation. A number of stage-specific genes and gene products encoded by gametocytes have been well studied in *P. falciparum* and *P. berghei* using transcriptomic approaches suggesting regulation of commitment genes in early gametocyte stages. About 200-300 transcripts have been identified to be expressed during the onset of gametocytogenesis with a small number reserved for later expressions. Notably, early gametocyte development (stage I-II) is associated with upregulated expression of *Pfs16* (Bruce et al., 1994), *Pfg27/25* (Olivieri et al., 2009), *SET*, *Pfmdv-1/Peg3*, *PfPeg4/etramp 10.3*, *Pfg14.744* and *Pfg14.478* (Eksi et al., 2005; Furuya et al., 2005; Silvestrini et al., 2005). Silvestrini and colleagues reported that the onset of gametocytogenesis is marked by protein trafficking following identification of *P. falciparum* gametocyte-exported proteins (*Pf*GEXPs) from 1427 proteins of highly enriched early-stage gametocytes using tandem mass spectroscopy. These putatively exported proteins were suggested to participate in RBC remodeling (Silvestrini et al., 2010). *Pf*GEXP5 encoded on chromosome 9 was identified by *in vitro* studies as an early gametocyte marker of sexual differentiation expressed at ring stage 14 h post-invasion (Tibúrcio, et al., 2015).

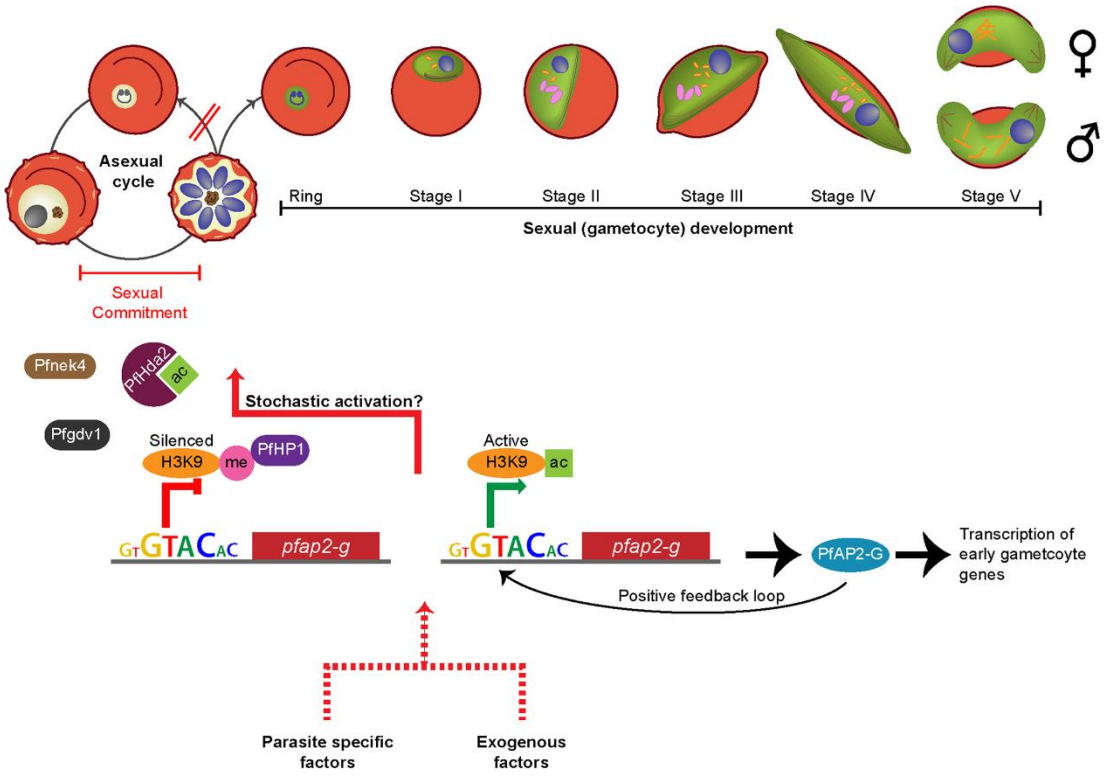


Figure 2.1: *Plasmodium* gametocytogenesis activation transcriptional switches. Commitment to exit asexual replication into sexual is induced by host environmental stimuli acting on parasite specific transcriptional programmes regulated by epigenetic histone post translational modifications. Plasmodium apetala 2 (Pfap2-g) locus is maintained at silenced state by PfHda2 and PfHP1. Removal of acetyl residues on histones and subsequent methylation of histone 3 lysine 9 (H3K9me3) promotes recruitment of PfHP1 maintaining the silenced state of PfAP2-G locus. Other proteins acting upstream of PfAP2-G to regulate gene expression during early gametocyte stages are Pfgdv1 and Pfnek-4. A positive feedback of activation stimulates expression of the master switch PfAP2-G initiating gametocyte formation through a series of developmental stages I-V (Adapted from Nilsson et al., 2015).

Investigations utilizing genome-wide transcriptomic approaches coupled with forward genetics have revealed an indispensable epigenetic control of conserved transcriptional regulators involved in cell fate decision of *Plasmodium* sexual development (Brancucci et al., 2014; Bechtsi & Waters, 2017) (Figure 2.1). The association of a repressive trimethylated lysine 9 histone protein 3 (H3K9me3) with heterochromatin protein 1

(HP1) regulates chromatin-mediated transcriptional changes on *Ap2-g* locus during developmental transitions. *Pf*HP1(*Pf*3D7_1220900) maintains and stochastically regulates a cascade of Apicomplexan Apetala 2 (ApiAP2) family proteins upon sensing the prevailing cues that transcriptionally activate the master switch protein, *Pf*AP2-G (*Pf*3D7_1222600) located on chromosome 12 (Kafsack et al., 2014; Sinha et al., 2014). The HP1-regulation of *P. falciparum* AP2-G is mediated by an upstream activator *Pf*GDV1 that antagonizes HP1-dependent gene silencing effects (Filarsky et al., 2018). Regarded as the "Holy Grail", conditional knockout and overexpression of inducible AP2-G validated its regulatory role by arresting commitment and priming gametocyte conversion 6 h post-induction in synchronous *P. berghei* cells, respectively (Modrzynska et al., 2017; Kent et al., 2018). These studies have allowed disentanglement of various ApiAP2 proteins that regulate gender- and stage-specific *Plasmodium* development including AP2-O2 and AP2-O3 in ookinetes.

Downstream AP2-G, another ApiAP2 protein AP2-G2 (*Pb*ANKA_103430) has been demonstrated to facilitate gametocyte maturation in *P. berghei*, but it is not a key player in gametocytogenesis (Sinha et al., 2014; Yuda et al., 2015). Besides *Pf*HP1, another epigenetic protein, class II histone decarboxylase 2 (*Pf*Hda2; *Pf*3D7_1008000) regulates sexual differentiation by spatially overlapping HP1, removing acetyl groups and keeping *Ap2-g* locus in a silenced state (Coleman et al., 2014). *In vitro* conditional depletion of *Pf*HP1 and/or *Pf*Hda1 in asexual stages activated a repertoire of transcriptional switches including *Pf*AP2-G and enhanced gametocyte production (Coleman et al., 2014; Modrzynska et al., 2017). The expression of a serine/threonine never-in-mitosis/*Aspergillus* (Nima)-related protein kinase, *Pf*nek-4, in a subset of asexual population undergoing schizogony has further been associated with regulation of onset and progression of sexual differentiation (Reininger et al., 2012). Gametocytogenesis is not only transcriptionally and epigenetically regulated but also controlled at posttranscriptional level. Two key RNA-binding proteins (RBPs) of Pumilio/FBF (Puf) family acting at posttranscriptional level of gametocytogenesis; *Pf*Puf1 (*Pf*3D7_0518700) and *Pf*Puf2 (*Pf*3D7_0417100) have been identified and suggested to

be translationally repressed for later stage expression (Miao et al., 2010; Shrestha et al., 2016). Whereas Puf2 acts as a repressor protein through interaction with 5' and 3' untranslated regions of sexual transcripts (Miao et al., 2013), the regulatory mechanism mediated by Puf1 remains unknown. The main molecular players involved in regulation of sexual differentiation have been recently deconvolved; however, other functional interactive proteins remain unresolved yet although efforts are underway to characterize their functions.

Lipid environment also plays a significant metabolic role during gametocytogenesis. Using genetic and pharmacological approaches, Bobenchik et al., (2013) indirectly demonstrated that the phospholipid biosynthetic pathway regulated by phosphatidylcholine methyltransferase (*Pf*PMT; *Pf*3D7_1343000) mediate structural and regulatory roles during parasite differentiation and plays an indispensable role in gametocytogenesis. Another protein associated with lipid metabolism implicated in gametocytogenesis is ATP-binding cassette subfamily G member 2 (*Pf*ABCG2; *Pf*3D7_1426500) expressed by female gametocytes (Tran et al., 2014). Additionally, whilst there is a great decrease in phospholipids abundance during the transitional stages of gametocytogenesis (~ 67%) following upregulation of putative lysophospholipase (*Pf*I1775W) by AP2-G (Tran et al., 2016a), the expression of putative phosphatidylserine synthase (*Pf*3D7_1366800) and dhCer synthase remained high throughout gametocytogenesis (Gulati et al., 2015). These lipidomic findings support the essential nature of phosphatidylserine and ceramide biosynthesis in gametocyte conversion and viability.

Soon after sexual differentiation and before peripheral circulation in the host, the immature gametocyte stages (I - III) sequester within the extravascular niches of less immune surveillance of bone marrow and spleen for ~10 days awaiting maturation. This evolutionary phenomenon has been well demonstrated by correlative studies conducted on anemic pediatrics in Mozambique and Malawi. High transcript copy numbers of mature gametocytes in bone marrow were associated with increased prevalence of

microscopically detected parasites (Aguilar et al., 2014; Joice et al., 2014). Similar findings have been reported using rodent parasite model *P. berghei* (De Niz et al., 2018). On maturation, stages IV - V gametocytes reversibly remodel *i*RBCs paving way into peripheral circulation, a process mediated by *Plasmodium* helical interspersed subtelomeric (PHIST) proteins and regulated by parasite cyclic AMP-dependent kinase A signaling and phosphodiesterase (PDE) (Ramdani et al., 2015; Dearnley et al., 2016; Warncke et al., 2016). Additionally, it has been suggested that gametocyte-induced host RBCs deformability through protein kinase A (PKA)-mediated phosphorylation of STEVOR (SubTElomeric Variable Open Reading frame) C-terminal allows persistence in peripheral circulation for days before mosquito uptake (Naissant et al., 2016). Just before transmission into mosquito vector, previous studies have reported 126 epigenetically regulated gametocyte genes differentially expressed in preparation for environment change, among them functioning in signaling, cell cycle and gene regulation (Ngwa et al., 2013; Ngwa et al., 2017)

By manipulating the host odor profiles, gametocytemic individuals relatively attract more female mosquitoes for blood acquisition compared to non-infected facilitating transmission (Busula et al., 2017; Stanczyk, Mescher, & De Moraes, 2017). This is in part due to emission of key biomarker volatile signatures of infection: 4-hydroxy-4-methylpentan-2-one (**1)**, nonanal **(2)**, toluene **(3)**, hexanal **(4)**, ethylbenzene **(5)**, and 2-ethylhexan-1-ol **(6)** (Figure 2.2) that favor mosquito attractions (De Moraes et al., 2018; Robinson et al., 2018).

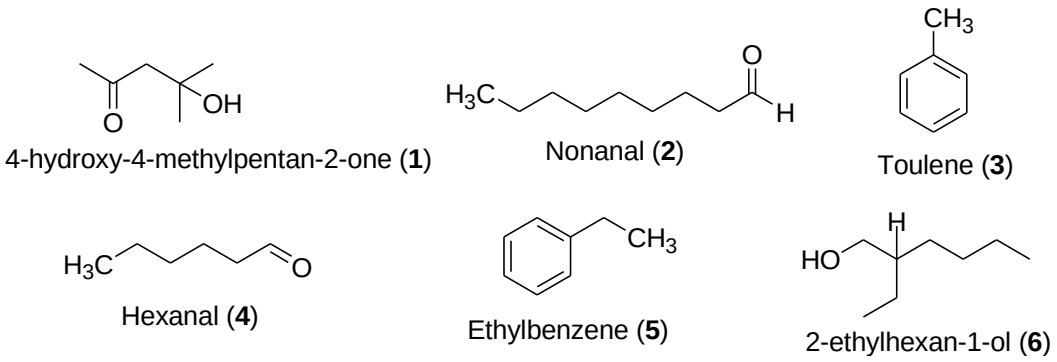


Figure 2.2: Chemical structures of human skin volatile metabolites identified during malaria infection.

2.1.2 Sporogonic development of *Plasmodium* parasites

2.1.2.1 Gametocyte activation: gametogenesis

Mosquito ingestion of quiescent stage V gametocytes triggers differentiation into micro- and macrogametes following a quick sensation of shift in pH (7.5 - 7.6) and temperature (19 - 21˚C for *P. berghei* and 24 - 34˚C for *P. falciparum*) within the midgut, a process facilitated by the presence of xanthurenic acid (XA) (Billker et al., 1998). The gametogenesis takes ~1 hour after blood meal uptake (Figure 2.3). The XA–induced activation of the quiescent gametocytes (G0 phase of cell cycle) stimulates influx of secondary messengers; Ca^{2+}, cyclic guanosine monophosphate (cGMP) and cGMP-dependent kinase (PKG) into gametocyte cytoplasm that facilitate fertilization (Muhia et al., 2001; McRobert et al., 2008). Furthermore, the activation is consequently accompanied by co-ordinated metabolic reprogramming for adaptation in midgut environment. Comprehensive metabolomic analyses have reported adaptive glutamine and high lipid requirements during sporogonic development (Srivastava et al., 2016). The egress from the enclosing RBCs into the midgut is aided by perforin-like proteases such as falcipin-2, plasmepsin II, PPLP-2 and expression of gametocyte-specific *Pf*g377 and *Pf*MDV-1/Peg3 essential for osmiophilic body formation (De Koning-Ward et al., 2008; Wirth et al., 2014).

2.1.2.2 Male exflagellation

Within the first 10 - 15 min post activation, each male gametocyte undergoes three rounds of rapid mitotic cell divisions and axoneme assembly yielding eight flagellated motile male (micro-) gametes through an exflagellation process (Janse et al., 1986). The male exflagellation is dominated by reversible protein phosphorylation events that rely on Ca^{2+}–dependent kinase *Pf*CDPK4 for genome replication (Billker et al., 2004); Doering et al., 2015) and atypical mitogen-activated protein kinase *Pb*MAP-2 for explosive release of the 8 flagellated microgametes (Tewari et al., 2005). A major adhesive protein *Pfs*230 is abundantly expressed on the surface of exflagellating microgamete to mediate binding onto sialic acids and glycophorin A of erythrocytes during exflagellation (Templeton et al., 1998). It has also been suggested that other signaling secondary messengers such as inositol triphosphate (IP3) and diacylglycerol (DAG) facilitate exflagellation (Martin et al., 1994). Through a systematic functional analysis of *Plasmodium* kinome, Tewari et al., (2010) established a putative serine/arginine-rich (SR) protein kinase (*Pb*SRPK) orthologous to *Pf*CLK4 that participate in exflagellation by regulating alternative splicing and cell cycle at M phase. Due to its unique development, it was intriguingly reported that knockout of a basal body spindle assembly-related protein *Pb*SAS-6 severely affects male gametogenesis, axoneme assembly, nuclear allocation, and perturbs fertilization and subsequent mosquito infection (Marques et al., 2015). Other proteins reported to functionally participate in male exflagellation include; *Pf*MDV-1 (Furuya et al., 2005), α-tubulin II (Kooij et al., 2005), *Pb*NEK-1 (Khan et al., 2005), *Pb*ppm1 (Guttery et al., 2014), actin II (Deligianni et al., 2011) and *Pfs*16 that encodes for an armadillo repeat motif (ARM) of flagellum apparatus (Straschil et al., 2010), a conserved nuclear protein SET (synonymous PHAPII, TAF-Iβ, I_2^{PP2A})(Pace et al., 2006), histone chaperone FACT (Laurentino et al., 2011) and putative homologue of CDC20/CDH1 (Guttery et al., 2012).

2.1.2.3 Macrogametogenesis

The female gametocyte possessing maternal translationally-repressed (TR) transcripts stabilized by a DEAD box RNA-binding helicase protein *Pb*DOZI (development of zygote inhibited; DDX6) (Mair et al., 2006) and Sm-like protein CITH (CAR-I in fly and Trailer Hitch) (Mair et al., 2010) differentiates into a single spherical female (macro-) gamete without DNA replication. A conserved TR-associated element U-rich RNA regulates translation by silencing either 3' or 5' untranslated regions (UTR) of *Pbs*28, *Pb*000245.02.0 and *Pbs*25 transcripts, respectively (Guerreiro et al., 2014). Furthermore, it has been suggested that interaction of DOZI with elongation factor eIF4E regulates translation of stored cytoplasmic proteins in female gametocytes (Tarique et al., 2013). Recently, a study by Bennink et al., (2018) identified a novel seven-helix stress granule protein (7-Helix-1) that was found to localize in female gametocytes and interacted with DOZI, CITH and PABP1 RBPs to regulate synthesis of macrogamete-specific proteins. Only on exiting the enclosing RBCs and differentiating into macrogametes, the stored DOZI-mediated TR proteins are translated activating expression of female gametocyte-specific surface proteins Pfs28/Pfs25 as well as transcription factor AP2-O, LCCL repertoire, CPW-WPC and plasmepsin IV (Yuda et al., 2009; Rao et al., 2016). Another RBP of Puf family *Pf*Puf2, albeit expressed in both micro-and macrogametes, further regulates TR proteins in macrogametocytes through mechanisms independent of DOZI and CITH participations (Miao et al., 2013) and perhaps involved in sex allocation. Elsewhere, knockout of *Pf*CDPK1 in asexual parasites impaired both female and male gametogenesis implying its key role in sexual differentiation (Bansal et al., 2018), characterized by 100% female gametes remaining in RBCs and absence of male exflagellation. In *P. berghei*, *Pb*MDV-1 has been reported to play a role in female gametocyte activation and its knockout resulted in 86% reduction in ookinete formation (Lal et al., 2009). Gametogenesis is orchestrated by spatial expression of surface proteins such as epidermal growth factor-like (EGF) domain-possessing proteins *Pfs*25/28, cysteine-rich proteins *Pfs*230 and *Pfs*48/45, and the LCCL-domain containing *Pf*CCp proteins that disappear after fertilization (Pradel, 2007).

2.1.2.4 Fertilization and ookinete development

The sexual differentiation produces gender-biased dimorphic gametes i.e. more macrogametes than microgametes (3:1) resulting into few numbers of infective ookinetes. Following gametogenesis, the microgametes actively search for the macrogametes for fertilization (~ 1 h) to produce diploid zygotes (~ 30 min) that meiotically transform into tetraploid motile banana-shaped ookinetes within 20-24 h post bloodfeeding (Kuehn & Pradel, 2010). High rates of meiotic recombination and genetic reassortment occur during fertilization resulting into distinct *Plasmodium* genotypes adaptive to varied host environments and facilitate evolution of drug resistance (Babiker & Walliker, 1997; Kooij & Matuschewski, 2007). The zygote formation occur through membrane fusion and involves proteins such as; Nima-related protein kinases, *Pf*nek-2 and *Pf*nek-4 (Reininger et al., 2009), cyclin G-associated kinase (*Pb*gak), protein kinase 7 (*Pb*pk7) (Tewari et al., 2010), Pfs48/45 (van Dijk et al., 2001), *Pb*CDPK1 (Sebastian et al., 2012) and microgamete generative cell specific 1 protein (GCS1) also known as hapless 2 (HAP2) (Liu et al., 2008). Using genetic mutants, it has been reported that development of ookinetes requires expression of a conserved regulator of clathrin uncoating *Pb*GAK (Tewari et al., 2010), ApiAP2 proteins AP2-O2, AP2-O3 (Modrzynska et al., 2017), Ca^{2+}/H^{+} exchanger (*Pb*CAX) (Guttery et al., 2013), *Pb*PPKL (Guttery et al., 2012), and *Pb*PPM2 (Guttery et al., 2014).

After formation, the polarized motile ookinetes glide out of the bloodmeal bolus through gut lumen and peritrophic matrix into the chitinous midgut epithelial cells facilitated by CDPK3, *Pb*PPKL, a microneme circumsporozoite thrombospondin related anonymous protein (*Pb*CTRP), a cGMP signaling pathway, Myosin A (Dessens et al., 1999; Billker, Lourido, & Sibley, 2009; Moon et al., 2009; Siden-Kiamos et al., 2011). Furthermore, ookinete gliding motility is aided by two conserved Asp-His-His-Cys palmitoyl-S-acyl-transferase proteins *Pb*DHHC2 and *Pb*DHHC3 without which ookinetes failed to invade mosquito epithelial cells (Santos et al., 2015; Hopp et al., 2016). Ookinetes have apical complex that possess microneme secretory organelles but to transverse the epithelium invading parasites require a *Shewanella*-like protein phosphatase (*Pb*SHLP1)(Patzewitz

et al., 2013), membrane attack ookinete protein (MAOP/PPLP3)(Kadota et al., 2004), PPLP5 (Ecker et al., 2007), and chitinase (*Pf*CHT1)(Vinetz et al., 1999). Midgut colonization by the invading ookinetes is associated with expression of proteins possessing antigenic and immunological properties including; enolase, von Willebrand factor A domain-related protein (WARP), subtilisin-like protease 2 (SUB2), cell traversal protein for ookinetes and sporozoites (CelTOS), secreted ookinete adhesive protein (SOAP), Pfs28, and Pfs25 (Angrisano et al., 2012).

2.1.2.5 Oocysts formation

Although with highly debated views, some ookinetes have been hypothesized to glide over the apical surface of midgut and traverse the epithelium at intercellular junctions into basal lamina where oocyst formation occur (Baton & Ranford-Cartwright, 2005). After crossing the epithelium and settling in the basal lamina, the ookinetes gliding motility is arrested and this trigger rounding up with reabsorption of apical complex into the cytoplasm to form multinucleated non-motile oocysts. Through *in vitro* studies, the ookinete-to-oocyst transformation has been reported to be triggered by the availability of bicarbonate and the complete shape induced by a range of nutrients but not dependent on basal lamina components (Carter et al., 2007). This is a fate stage that determines whether or not a successful invasion establishes for subsequent transmission as large numbers of ookinetes are lost to intensely activated mosquito immune responses. Oocyst formation is the longest stage of *Plasmodium* development in the mosquito midgut variably taking 10 - 12 days (Figure 2.3) and just like the other stages, the developmental stage is regulated by a number of genes and proteins. Due to the hostile immune responses, expression of oocyst capsule protein, *Pb*Cap380, and a capsule-associated protein (*Pb*Cap93) protects the developing oocysts from immune attacks and their deletion resulted in normal numbers that were gradually cleared or few sporozoites formed (Srinivasan, Fujioka, & Jacobs-Lorena, 2008; Sasaki et al., 2017). Another study by Gissot et al., (2008) pointed out that the formation of oocysts critically involves a DNA-binding regulator high mobility group nuclear protein *Py*HMGB2. It was demonstrated that disruption of HMGB2 resulted into normal gametocytes but

phenotypically impaired oocysts with ~30 downregulated genes. Through genetic deletions, a phosphatase *Pb*PPM5 was implicated essential in oocysts formation as mutant parasites resulted into viable ookinetes but yielded reduced numbers of fully formed oocysts (Guttery et al., 2014). Tewari et al., (2010) further demonstrated that signal transduction kinases *Pb*gak, *Pb*pk7 and a putative CDPK-like kinase (cdlk; PBANKA_101980) regulate sporogonic development with genetic mutants producing <10% oocysts. Detailed gene deletion experimental studies have demonstrated that female gametocyte-inherited limulus clotting factor C, Coch-5b2 and LglI (LCCL)/lectin adhesive-like proteins (LAP) are expressed during *P. berghei* oocysts development. It was demonstrated that among the six *lap* genes encoded by *Plasmodium*, targeted disruption of *Pblap1/CCp3/SR*, *Pblap2/CCp1*, *Pblap4/CCp2* and *Pblap6/CCp4* resulted in abnormal oocysts formation with immature nuclear organization, reduced sporozoites numbers, and suggested to regulate cell cycle events (Raine et al., 2007). Elsewhere, Hart et al., (2016) reported that *P. yoelii* mutants of coenzyme A biosynthetic pantothenate kinase genes, *PyPanK*1 and *PyPanK2*, displayed normal asexual and sexual replication but defects in ookinetes, oocysts and sporozoites formation. This demonstrates regulatory utilization of pantothenate in mosquito stages. Another protein expressed in oocysts during their development is *Pb*IMC1a, involved in the formation of inner membrane complex (IMC) of microtubules and its disruption resulted in deformed sporozoites with defective motility (Khater et al., 2004). A recent study reported that a genetic deletion of plant P-type cyclin, (CYC3), resulted into defects in oocysts development with abnormal budding and sporozoites formation, therefore required for endomitotic oocysts development (Roques et al., 2015).

2.1.2.6 The egress and maturation of infective sporozoites

Within a span of 8 - 14 days post activation, the oocysts undergo sporogony phase resulting in the formation of hundreds of haploid sporozoites that upon oocysts rupture invade and lodge within the salivary glands (Smith *et al.*, 2014). The crescent-shaped sporozoite egress from oocysts capsule into hemocoel requires a proteolytic activity of parasite cysteine protease known as egress cysteine protein 1 (ECP1/SERA8) (Aly &

Matuschewski, 2005). On release, the sporozoites are thought to passively migrate through the hemocoel to invade the salivary glands and awaiting inoculation into new hosts ~ day 14 - 21 post activation. Although the proteins that aid in sporozoites development and maturation appear to recur in preceding ookinetes and oocysts stages, sporozoites-specific proteins have still been identified and functionally characterized. In a comprehensive study of *Plasmodium* kinome, Tewari et al., (2010) established that kinases PBANKA_040940 and sucrose nonfermenting (Snf)1-related/AMP-activated kinase, *Pbkin*, to regulate sporozoites egress as the parasite mutants resulted in 90 - 95% reduction of salivary gland sporozoites and the remaining numbers failed to establish in C57BL/6 mice. Sporozoites formation also requires lipids, and thus deletion of type II fatty acids biosynthetic (FAS-II) genes *fabI* and *fabB*/*F* did not either affect asexual stages or oocysts formation but abolished sporozoites development (L. et al., 2014). Further detailed analysis of sporozoites proteome by Lasonder et al., (2008) revealed a number of proteins distinctly specialized for sporozoites maturation, motility, and infectivity. These include; circumsporozoite surface protein (CSP) whose N- and C-terminal repeat domains were functionally found to aid in sporozoites formation and maturation (Ferguson et al., 2014), CTRP, also expressed in ookinetes for motility aid sporozoites to glide in addition to thrombospondin-related anonymous protein (TRAP) that through binding onto mosquito saglins invade salivary glands (Ghosh et al., 2009), CelTOS, sporozoite microneme protein essential for cell transversal 1 and 2 (SPECT1, SPECT2), myosin A, MyoA Tail Domain interacting protein (MTIP), cysteine repeat modular proteins CRMP1 and CRMP2 previously found to aid in sporozoites maturation (Thompson et al., 2007), merozoite antigen erythrocyte binding ligand (MAEBL), MAL8P1.66, and PF14_0435. To infect the salivary glands, it was recently demonstrated that an endogenous protein *Pb*LIMP (PBANKA_0605800) derived from a peculiar gliding phenotype following epitope tagging was crucial for sporozoites gliding motility (Santos et al., 2017). The upregulated expression of eukaryotic translation initiator factor 2 alpha (eIF2α) kinase (IK2) in invading sporozoites (Zhang et al., 2010) is regulated by RBP Puf2 to prevent premature sporozoites transformation in *P. berghei* (Müller et al.,

2011). Other sporozoites-specific proteins expressed during development include; upregulated-in-oocysts sporozoites protein 3 (UOS3/TREP/S6)(Steinbuechel & Matuschewski, 2009), protein tyrosine phosphatase-like A (PTPLA) (Guttery et al., 2014) and cyclin-dependent-like kinase (Tewari et al., 2010). During blood feeding on a new host, the infective mature sporozoites are injected together with anticoagulant saliva into host bloodstream, closing the current transmission cycle and starting a new within-vertebrate host cycle.

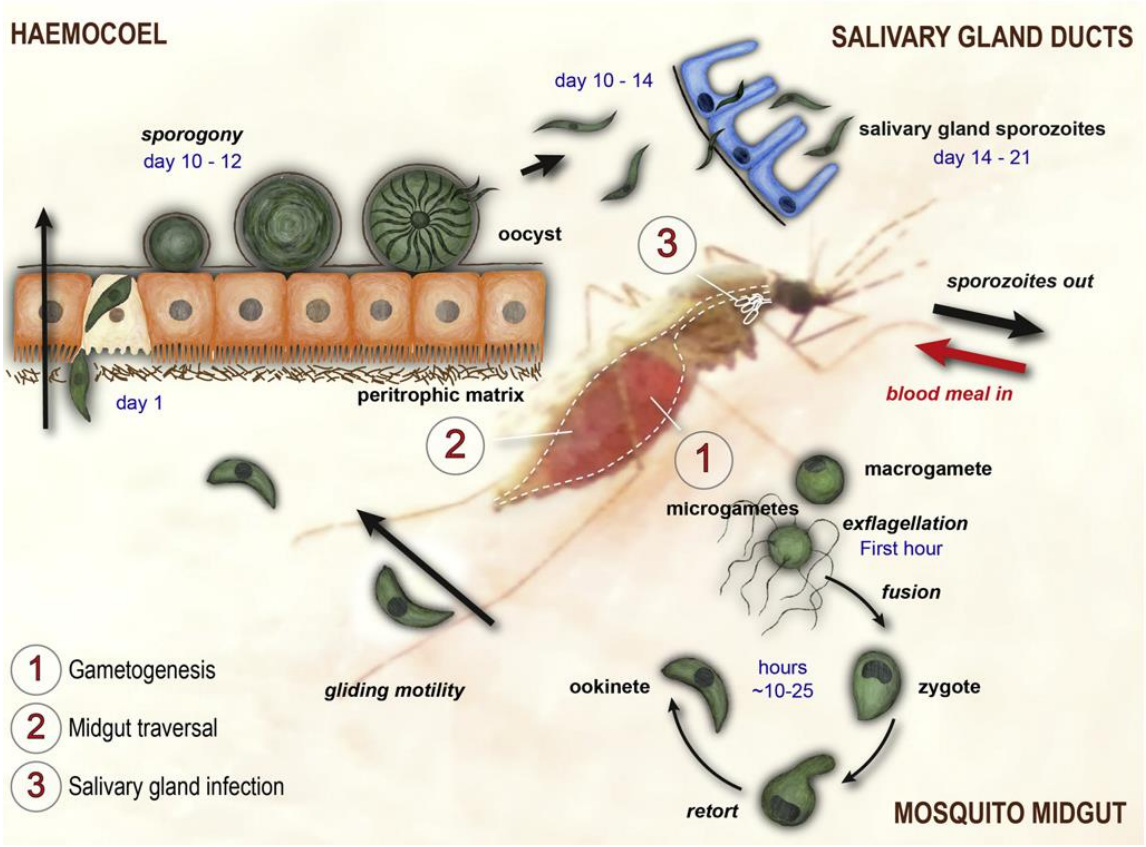


Figure 2.3: Sexual development of malaria parasites in mosquito midgut. Late-stage V Plasmodium gametocytes are ingested by female mosquitoes during blood feeding. On reaching the midgut, due an abrupt change of environment, the haploid parasites egress from infected erythrocytes, differentiate into micro- and macrogametes that fuse to form a zygote. The zygote undergoes meiotic replication transforming into a motile ookinete that glide and transverse midgut epithelium cells into basal lamina to form oocysts. The oocysts undergo extensive developmental events within 10 - 14 days resulting into several thousands of sporozoites. The sporozoites egress from the oocysts and infect the salivary gland acinar cells before entering the ducts of the salivary glands awaiting

inoculation into human host during the subsequent blood feeding (Adapted from Angrisano et al., 2012).

2.1.3 Dynamics of *Plasmodium* development in the mosquito and transmission success

Successful parasite development in the mosquito midgut and subsequent transmission outcome involves an intricate tripartite *Plasmodium*-mosquito-microbiota crosstalk with "invade and conquer" mechanisms mediated by a number of conserved interactive proteins (Figure 2.4) (Sreenivasamurthy et al., 2013; Bennink et al., 2016; Romoli & Gendrin, 2018). While the parassite strives to invade the mosquito cells and complete its lifecycle, the mosquito immune responses block its development even though some proteins are implicated in promoting parasite escape and survival.

2.1.3.1 Agonistic midgut-ookinete molecular interactions in invasion of *Plasmodium*

It is now evident that invasion of mosquito midgut by ookinetes occurs through a multi-route kind of receptor-ligand mediated molecular interactions involving protein-protein, carbohydrate-protein and lipid-protein adhesions. By expressing micronemal invasive factors CelTOS, CTRP, WARP, SOAP, MAOP, PPLP3-5, and chitinase (CHIT1), and surface proteins Pfs25/Pfs28, ookinetes interact with highly glycosylated oligosaccharide residues on the microvillar lining of mosquito midgut (Wilkins & Billingsley, 2001). The ensuing notion about midgut-ookinete interactions was classically demonstrated using a 12-amino acid peptide selected from a phage display library, salivary gland and midgut peptide (SM1). The dodecapeptide SM1 was found to bind the luminal side of midgut epithelium blocking *P. berghei* ookinetes invasion (Ghosh et al., 2001). *P. gallinaceum* ookinetes were found to adhere to carbohydrate ligands on microvillar wall of midgut epithelium (Zieler, Nawrocki, & Shahabuddin, 1999). Additionally, using snake venom phospholipase A2 blocked midgut-ookinete interactions (Zieler et al., 2001), a phenomenon further replicated in two separate studies upon generation of a transgenic mosquito expressing SM1 or phospholipase A2 (Ito et al., 2002; Moreira &

Jacobs-Lorena, 2003). Using these evidential findings as baseline, a number of studies characterizing vector-parasite interactions during *Plasmodium* infection have been conducted. For instance, despite not considered an indispensable factor for successful ookinete-to-oocyst development *in vitro* (Carter et al., 2007), RNA*i*-mediated depletion of mosquito basal laminin showed its requirement for midgut-ookinete association and its absence reduced oocysts numbers (Arrighi et al., 2005). Another study by Rodríguez et al., (2007) established that surface protein *Pvs*25 on *P. vivax* ookinetes interacted with mosquito calreticulin. Just like in erythrocytic plasmodial invasions, ookinetes utilize sulfated chondroitin glycosaminoglycans (GAGs) for midgut invasions (Dinglasan et al., 2007), a similar observation made for sporozoites in salivary gland (Armistead et al., 2011). RNA*i*-mediated peptide-*O*-xylosyltransferase (*AgOXT1*) gene silencing and targeted inhibition with small molecule GAGs mimetic resulted in 95 - 99% reduction of midgut colonization (Dinglasan et al., 2007; Mathias et al., 2013). Lavazec and colleagues demonstrated that antibodies targeting mosquito carboxypeptidase B inhibited oocyst development suggesting their agonistic interation with invading ookinetes (Lavazec et al., 2007). Moreover, RNA*i*-mediated deletion targeting mosquito transmembrane scavenger receptor protein Croquemort SCRBQ2 homolog blocked ookinete midgut colonization and oocysts development by 62.5% (González-Lázaro et al., 2009). Recent studies have discovered that a conserved fibrinogen-related protein FREP1 on peritrophic matrix anchors ookinetes to facilitate invasion into epithelium and its depletion, use of anti-FREP1 antibody and compounds results in reduced oocysts formation (Zhang et al., 2015; Niu et al., 2015; Dong et al., 2018). Microvillar proteins alanyl aminopeptidase N1 (*Ag*APN1) and myosin also facilitate midgut invasion and blockade with antibodies inhibited *P. berghei*, *P. vivax* and *P. falciparum* oocysts development (Dinglasan et al., 2007; Lecona-Valera et al., 2016).

A constitutively expressed mosquito epithelial serine protease (*Ag*ESP) on submicrovillar region was established to agonistically promote *P. berghei* and *P. falciparum* infections. Intriguingly, *Ag*ESP silencing reduced midgut invasions and transcriptionally repressed expression of gelsolin that regulate actin remodeling of

midgut cells (Rodrigues et al., 2012). Matrix metalloproteases (MMPs) proteolytically degrade extracellular matrix collagen IV and participate in wound healing process in arthropods and also modulates innate defenses. In mosquitoes, through dsRNA gene silencing, *Ag*MMP1 was implicated in agonistic protection effect against ookinetes and facilitating oocysts development (Goulielmaki & Loukeris, 2014). Among the defense mechanisms deployed by mosquitoes against invading ookinetes is melanization. Melanization of insect midgut is catalyzed by prophenoloxidase (PPO) from 3,4-dihydroxyphenylalanine (L-DOPA) metabolic conversions following microbial invasions. Serpins, and particularly Serpin-2, have been reported to negatively regulate PPO activation and consequently promoting *Plasmodium* infection in *An. gambiae* (Michel et al., 2006). Despite recently showing opposing effects in *An. albimanus* (Simões et al., 2017), functional knockout of C-type lectins CTL4 and CTLMA2 in *An. gambiae* resulted in 97% and 53% melanization of invading ookinetes, respectively (Osta et al., 2004). This observation suggests an agonistic protective effect of the C-type lectins against melanostic killing effect. Although not directly interacting with ookinetes, apolipophorins APOII/I (Mendes et al., 2008) and ApoLp-III (Dhawan et al., 2017) also facilitate midgut invasion as their silencing reduced oocysts by 7.7 fold and induced NOS that mediate ookinete killing contrary to previous findings reported in *An. gambiae* G3 by Gupta et al., (2010).

2.1.3.2 Midgut epithelium defense responses during ookinete invasion

Cell transversal-mediated invasion of mosquito midgut epithelium into basal labyrinth by ookinetes is associated with intense multifaceted immunological responses triggered by apoptotic signals, inducible stress responses and ookinete surface proteins. It has been shown that, following activations, about 100-fold loss of ookinetes occur marking a major population bottleneck loss of *Plasmodium* life cycle (Smith et al., 2014). Responsive genes and proteins have been identified and functionally characterized using reverse genetics over the past few decades, demystifying the possible underlying immunological mechanisms of mosquito refractoriness (Christophides et al., 2004; Vlachou et al., 2005; Baton et al., 2008; Severo & Levashina, 2014). Activation of

caspase-3-like protease by invading ookinetes not only results in cell death, but also triggers apoptotic-mediated detonation of immune responses theorized as “time-bomb theory” that kill several if not all the ookinetes (Han et al., 2000; Baton & Ranford-Cartwright, 2004; Moreno-García et al., 2014). This apoptotic damage is characterized by spontaneous release of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) of Jan kinase / signal transducers and activators of terminal kinase (JAK/STAT) pathway, extensive DNA fragmentation, protrusion of invaded cells to midgut lumen, and loss of microvilli. Furthermore, the activation is hallmarked by induction of hemocyte-specific responsive factors thioester-containing complement-like protein TEP1, fibrinogen-like immunolectin FBN9, Nimrod, Eiger, lipophorin receptor, among others that modulate cascades of integrated immune regulatory signaling pathways (Lombardo et al., 2013).

Discovered early in 2000s, the mosquito complement-like TEP1 and a homolog of human complement C3b, recognize and binds onto ookinetes surface mediating lytic or melanostic killing of ~80% *P. berghei* ookinetes (Blandin et al., 2004). Activation of TEP1 is stabilized by the formation of a heterodimeric complex of two leucine-rich repeat (LRR) domain proteins *Anopheles Plasmodium*-responsive leucine-rich repeat 1C (APL1C) and leucine-rich repeat immune 1 (LRIM1) (Fraiture et al., 2009), and regulated by *Rel1* and *Rel2* of immune deficient (imd) signaling pathway (Riehle et al., 2008). It has been demonstrated that RNA*i*-mediated *TEP1* gene silencing increased oocysts densities five-fold in susceptible and resistant mosquitoes whilst its transgenic expression did not warrant elevated resistance to *Plasmodium* infection albeit its endogenous antiparasitic effects (Blandin et al., 2004; Volohonsky et al., 2017). A just-in-time parallel two-step epithelial tyrosine nitration response against the transversing ookinetes induces production of heme peroxidase 2 (HPX2) and NAPDH oxidase 5 (NOX5) that label the ookinetes surface for TEP1-dependent killing (Kumar et al., 2004; Oliveira et al., 2012). Deposition of TEP1 on ookinete surfaces is further suggested to be regulated through an analogous mammalian convertase-like activity mediated by two noncatalytic clip domain serine proteases (CLIPs), SPCLIP1 and CLIPA2, regulating positively and negatively, respectively. Another protein that influences TEP1-mediated

lysis of ookinetes is innexin AGAP001476 that through chemical blockade increased oocysts numbers and infection prevalence, accompanied by repression of *TEP1* levels (Li et al., 2014). Following ookinetes killing, the dead parasites are cleared from the host tissues through actin-based mechanisms (Shiao et al., 2006).

As part of the primitive insect innate defenses, the induced oxidative stress on midgut epithelial cells by tissue damage, *Plasmodium* glycosylphosphatidyl inositol (*Pf*GPI) and human blood components transcriptionally activates production of reactive oxygen and nitrogen intermediate species (RNOS), and NO (Peterson et al., 2007; Luckhart et al., 2015). These wound-induced epithelial responses were reported to be activated by AP-1 transcription factor Fos and transglutaminase 2 (TGase2) (AP-1/Fos-TGase2) axis as silencing negatively impacts infection resistance against *P. falciparum* (Nsango et al., 2013). Conversely, the anticipatory-fed blood components including insulin, transforming growth factor beta 1 (TGF-β1), hemozoin, growth hormones, and cytokines further induce synthesis of ROS abrogating *Plasmodium* development (Pakpour et al., 2013). The generated midgut ROS interchangeably activate conserved kinases; mitogen-activated protein kinases (p38 MAPK), c-Jun N-terminal kinase (JNK), extracellular-signal regulated kinase (ERK)), and phosphatidylinositol-3-kinase (PI3K)/Akt of insulin/insulin-like signaling (IIS) pathways (Surachetpong et al., 2011; Luckhart et al., 2015), regulating protein kinase C (PKC)-dependent epithelial barrier integrity, nitration, and melanostic encapsulation (Kumar et al., 2003). Blockade of iNOS and regulatory signaling effectors through gene knockdown doubles oocysts densities possibly due to immunomodulatory heme peroxidase (HPX15)/dual oxidase (Duox) system that prevents activation of epithelial immune responses (Kumar et al., 2010). In fact, previous studies have demonstrated, through gene silencing, that JNK-regulated expression of basal key detoxification enzymes: superoxide dismutase (SOD), oxidation resistance 1 (OXR1), catalase, glutathione peroxidase (Gpx) antagonize the generated reactive species by facilitating parasite survival (Jaramillo-Gutierrez et al., 2010).

The mosquito midgut is rich in well-adapted symbiotic microbiota that also innately contribute towards *Plasmodium* defenses by either secreting effector antimicrobial peptides (AMPs) with antiplasmodial effects or effectively modulating immune regulatory signaling pathways; nuclear factor kappaB (NF-*k*B)-dependent Toll and imd and JAK/STAT (Dong et al., 2009; Cirimotich et al., 2011). Notably, Gram-positive and Gram-negative bacteria; *Elizabethkingia meningoseptica*, *Asia bogorensis*, *Enterobacter* spp, *Pantoea stewartii*, *Burkholderia* spp., *Aeromonas* spp., *Serratia* spp., *Chryseobacterium* spp, *Ewingella* spp., *Cedecea* spp., *Comamonas* spp., among others and fungi inhabit the midgut as commensals (Boissière et al., 2012). Through the engagement of Rel1/toll-dependent and Rel2/imd-associated receptors of non-self peptidoglycan recognition proteins PGRPLA, PGRPLC and PGRPLD, induces secretion of antimalarial AMPs gambicin, defensin, cecropin B, romidepsin (Vizioli et al., 2001; Meister et al., 2009; Gendrin et al., 2017; Saraiva et al., 2018) in addition to regulating the peritrophic matrix barrier integrity (Rodgers et al., 2017; Song et al., 2018). Effector molecules of the imd pathway include; TEP1, FBN9, leucine-rich repeat domain containing protein 7 (LRRD7). dsRNA knockdown of either the PGRPs or AMP effector genes decreases microbiota loads and consequentially increasing susceptibility to *Plasmodium* infection, observations made from imd antagonists like *caudal* (Clayton et al, 2013).

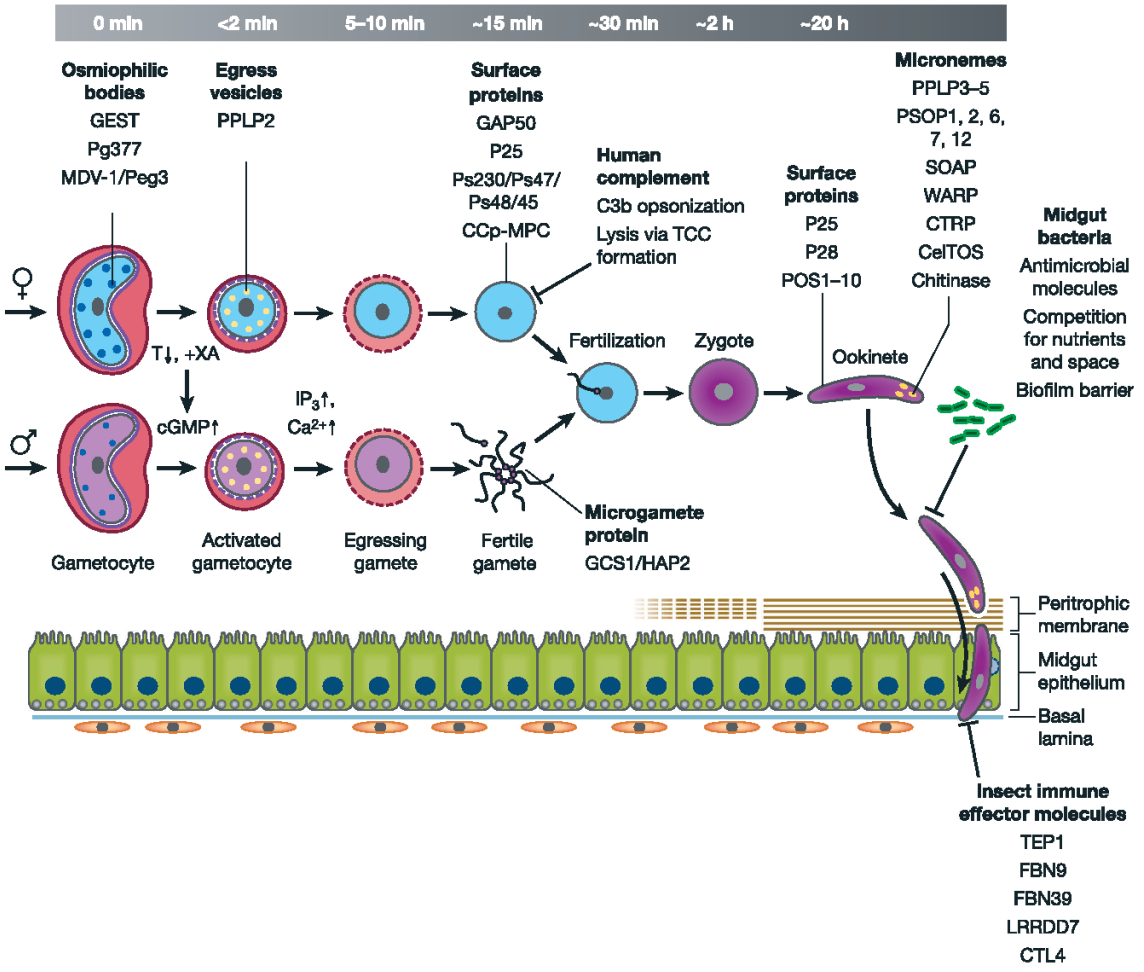


Figure 2.4: Functional regulatory proteins involved in *Plasmodium*-mosquito interaction during sporogony. Developmental progression of sexual gametocytes following midgut activation by induced xanthurenic acid (XA) and reduced temperature is orchestrated by expression of stage-specific gene products. Expression of Pg377, male development-1(MDV-1), *Plasmodium* perforin-like protein 2 (PPLP2) and gamete egress and sporozoite traversal (GEST) promote egress from enclosing RBCs through formation of osmiophilic bodies. Following activation, the male gametes under the influence of Ca^{2+}, inositol triphosphate (IP3), and cyclic guanosine monophosphate (cGMP) undergo exflagellation to form fertile male gametes expressing generative cell specific 1 (GCS1/HAP2). Notable surface proteins expressed on female gametes following activation of translationally repressed transcripts are Pfs25, Pfs48/45, Pfs230, P47, CCp-based multiprotein complex (CCp-MCP), and glideosome-associated protein (GAP50). The male and female gametes fuse to form a zygote that within 20-24 h differentiates meitocally into a motile banana-shaped ookinete. The ookinete expresses

surface proteins such as p25, p28, putative ookinete surface-associated proteins (pos1-10), and apical complex micronemes secreted ookinete adhesive protein (SOAP), von Willebrand factor A domain-related protein (WARP), chitinase, circumsporozoite and thrombospondin-related adhesive protein-related protein (CTRP), Cell-traversal protein for ookinetes and sporozoites (CelTOS), putative secreted ookinete proteins PSOP1,2,6,7,12, and *Plasmodium* perforin-like proteins 3/4/5 (PPLP3-5) that aid in cell transversal and midgut invasion. The sexual development in mosquito is counteracted by immune responses ranging from complement-like factors to microbiota-dependent antimicrobial peptides. The effector immune molecules acting at epithelial region include; leucine-rich-repeat domain-containing protein 7 (LRRDD7), thioester-containing protein 1 (TEP1), fibrinogen immunolectin 9/39 (FBN9/39), and C-type lectin 4 (CTL4)(Adapted from Bennink et al., 2016).

2.1.3.3 Parasite-induced immune evasion mechanisms

For the invading parasites to successfully complete their lifecycle and get transmitted, *Plasmodium* has evolved ways to outsmart the hostile mosquito immune defenses in the midgut. First, through the expression of 6-cysteine (6-cys) protein Pfs47/Pb47 located on chromosome 13 adjacent to Pfs48/45 that disrupt JNK signaling pathway and its subsequent activation of HPX2- and NOX5-mediated epithelial nitration (Molina-Cruz et al., 2013; Ramphul et al., 2015; Ukegbu et al., 2017). Compared to wild-type parasites that successfully invaded, knockout of Pfs47 resulted in efficient elimination following JNK-mediated mosquito cell apoptosis through activation of *forkhead box O* (FOXO), caspase *(CASP)-L1* and other downstream effector caspases such as *CASP-S2* (Ramphul et al., 2015). Absence of epithelial nitration from HPX2 and NOX5 that precludes release of TEP1 from the hemocyte microvesicles inhibits ookinete binding and killing.

Another immune evasion strategy deployed by *Plasmodium* is through induction of insulin/insulin-like signaling (IIS) pathway and synthesis of insulin-like peptides (ILPs) (Pietri et al., 2016). dsRNA knockdown of insulin-like peptides ILP4 and ILP3 induced early (1-6 h post infection) and later (24 h) expression of effector immune genes, respectively, and positively correlated with reduced oocysts numbers. The dampening effect of immune responses through alteration of mosquito ILP expression patterns is a

strategy facilitating *Plasmodium* development. Elsewhere, it has been demonstrated that by co-opting human plasminogen captured by ookinete surface enolase and coating themselves with complement regulator factor H, facilitate hijacking of the killer complement system (Ghosh et al., 2011; Simon et al., 2013).

2.2 Human-mosquito *Plasmodium* transmission

Successful transmission of *Plasmodium* parasites from infected humans to mosquitoes and subsequent inoculation into uninfected hosts involves (i) uptake of developmentally-arrested intracellular gametocytes by female *Anopheles* mosquitoes during bloodmeal acquisition, (ii) sporogonic development in mosquito midgut and (iii) inoculation of infective sporozoites into human bloodstream (Beier, 1998). Intrinsic appetitive stimuli as well as manipulative effects of *Plasmodium* infection drive female mosquitoes to search for bloodmeals from proximate human hosts. The long-range search is guided by olfactory-response chemical cues from the host's skin volatile metabolites and exhales (Takken & Knols, 1999). The ingested blood is meant to sustain protein requirements and development of eggs for lineage progression. However, as the mosquitoes acquire bloodmeals, they deliberately inject infectious sporozoites in anticoagulant saliva into human bloodstream.

Epidemiologically, global transmission of clonally variant malaria parasites is complex, regionally and seasonally dynamic, and non-uniformly distributed, with particular geographical regions exhibiting low transmissions while others experience high intensities (Figure 2.4). This variation is largely associated with relative abundance of vector-competent *Anopheles* mosquitoes and human exposure risks (Guelbéogo et al., 2018). In the year 2019, out of the estimated 228 million cases and 405,000 deaths reported globally in WHO Malaria Report, the WHO African region contributed 93% while South East Asia recorded 3.4% and East Mediterranean region registered 2.1%. Sadly, although tremendous changes in parasite population structure and progressive reduction in transmission have been witnessed over the last decade, the disease burden is still high among school-going children within the age bracket of 2-10 years irrespective

of the geographical malaria fringe (Noor et al., 2014; Nkumama, O'Meara, & Osier, 2017). Restricting the focus into sub-Saharan Africa where most malaria cases are reported, the varying transmission patterns have further been attributed to multiple interactions of non-genetic or environmental as well as evolutionary genetic influences on mosquito vector populations and circulating *Plasmodium* parasites (Cohuet et al., 2010; Lefevre et al., 2017). In fact, the tropical climatic conditions that prevail across sub-Saharan Africa coupled with emerging rates of industrialization and development, promotes *Anopheles* mosquito breeding, longevity as well as adaptations through species speciation and chromosomal inversions (Fontenille & Simard, 2004; Fouet et al., 2018). The ensuing phenomena of species divergence could also significantly influence *Plasmodium* susceptibility and insecticide resistance. Additionally, the constant change of treatment regimens particularly sulfadoxine/pyrimethamine (SP) and artemisinin combined therapies (ACTs) as a result of drug failure complicates transmission cycle with variant parasite clones from varied genetic backgrounds (Hill & Babiker, 1995; Gbotosho et al., 2011).

At human host level, when exposed to similar risk factors, individuals disproportionately show differential susceptibility to mosquito bites and infection establishment. This suggests that the substantial heterogeneity in disease transmission dynamics is variably driven by the immune effects and human genetics although undetectable gametocyte carriage quantitatively sustains transmission in various regions (Bousema et al., 2011; Busula et al., 2017; Grignard et al., 2018). Again, age-dependent gametocyte density is also evidently possible from immune responses and influences transmission rates as children have high gametocytemia while adults serve as infection reservoirs (Bousema et al., 2004; Ouédraogo et al., 2010). Classically, in cross-sectional epidemiological surveys conducted in regions of varying transmission intensities in Burkina Faso and Kenya, differential mosquito infectivities were recorded with more children than adults transmitting parasites to mosquitoes while the adults received more bites to balance contribution in infectious reservoir (Gonçalves et al., 2017). A number of epidemiological model studies highlight a close relationship between host infectivity to

mosquito vectors and relative gametocyte densities, albeit quite varied due to gametocyte dynamics from different drug policies and vector control interventions (Drakeley et al., 2006; Ross, Killeen, & Smith, 2006). While the overall notion is that high gametocyte density promotes mosquito infectiousness, surprisingly it might not necessarily hold true as asymptomatic individuals with low-density infections (< 5,000 gametocytes/mL) were found to highly transmit (Coleman et al., 2004; Schneider et al., 2007; Bousema et al., 2014). This scenario creates a gap in knowledge of dynamic natural infectivity differences between symptomatic and asymptomatic individuals underscoring the limited understanding of malaria transmission. It is generally believed that as transmission intensity declines following malaria interventions gametocyte carriage becomes prevalent, creating malaria hotspots in low transmission regions. In fact, mathematical model fitted to epidemiological data collected from Dielmo, Senegal (1990-2008) showed that the proportion of infectious mosquitoes remained relatively high following interventions, suggesting equivocal efficiency in increased and sustained transmission due to high gametocyte densities (Churcher et al., 2015). Elsewhere, in an attempt to explain the high *Plasmodium* transmission rates in low than high transmission regions, Rono et al., (2018) reported that for the parasites to be transmitted, they evolutionary invest more through upregulation of AP2-G and HDA1 to generate more gametocytes accompanied by reduction of asexual replication processes.

Historically, the relative transmission dynamics between individuals was first modeled by Ronald Ross in 1900, soon after the discovery of malaria parasites in mosquitoes, and later advanced into Ross-MacDonald model to measure epidemiological transmission intensity (Macdonald, 1957; Smith et al., 2012) as outlined in the equation below. The initial Ross model put into consideration the contribution of human biting rates from mosquitoes and incidence of malaria from larval densities. Following Ross' ideas, George MacDonald suggested that several entomological bionomics including survival (longevity), biting frequency, human preference, and parasite extrinsic incubation period lacked for transmission to occur at unit time. By factoring in the Ross' parameters into his, Ross-MacDonald model of malaria transmission dynamics was formulated in 1957.

$$R_0 = \frac{ma^2bce^{-\mu T}}{r\mu}$$

Where parameters;

R_0 refers to the basic reproduction rate defined as the number of infections generated from infective individual in an immunologically naïve host population

m is the vector-host ratio defining the anopheline abundance in relation to humans

a refers to the mosquito human biting rate

b is the proportion of infective bites that leads to an infection in humans

c is the proportion of mosquito bites on infected humans that results in an infected mosquito

e is the natural logarithm

T refers to the parasite's extrinsic incubation period

μ is the daily mortality rate of mosquitoes

r is the recovery rate of humans from infection

The model holds that, for malaria transmission to occur, R_0 should be more than 1 ($R_0>1$) while elimination requires $R_0 <1$. Since the equation is linear, a significant reduction of mosquito populations through vector control, that also impact longevity, results into ~80% decrease in transmission following a decline in the number of human-vector contacts and proportion of infective insects. For this reason, vector control targeting adult mosquitoes is perceived to largely disrupt malaria transmission despite leaving the contribution of larval productivity.

Due to hematophagic behavior of mosquitoes, entomological inoculation rate (EIR), a product of mosquito biting frequency (*a*) and relative proportion of infected salivary glands (*c*), quantitatively assesses the transmission intensities and has become a common parameter in transmission foci dynamics. This has been made possible by combinatorial deployment of human landing collections coupled with enzyme-linked immunosorbent assay (ELISA) or PCR-based techniques targeting sporozoite's circumsporozoite protein (CSP), effectively quantifying the magnitude of malaria regionally and the impact of

vector control (Kilama et al., 2014). In Africa, it is estimated that annual EIR varies from 1 < to > 1000 per individual (Trape & Rogier, 1996). Although not every mosquito bite is necessarily infectious, it is assumed that the number of bites an individual receives contributes to EIR that correlate with parasite transmission and prevalence (Beier et al., 1999). However, EIR varies seasonally and annually due to varying mosquito abundance and extrinsic parasite incubation times as well as gametocytemic levels that differentially attract mosquitoes (Lacroix et al., 2005; Gadalla et al., 2016; Busula et al., 2017). Conversely, Churcher and co-investigators showed that transmission of malaria parasites is highly dependent on mosquito infection density, meaning that heavily infected salivary glands are likely to transmit malaria to human hosts irrespective of immunity levels (Churcher et al., 2017). As mosquitoes tend to bite, in most cases, at night and great investment is required for transmission by gametocytes, Schenider and co-investigators using mouse model parasite *P. chabaudi* showed that *Plasmodium* might have adaptively evolved to match the vector rhythms where mosquito infectivity by gametocytes was found to be twice at night. This was suggested to amplify sporozoites numbers by ~ 4-fold for day-time feeders and that gametocytes may be less infective at day time (P. Schneider et al., 2018), coinciding with mosquito behavioral evolution from vector control interventions. Convincingly, if malaria elimination from endemic geographical settings is feasible, then the discovery of transmission reducing interventions (TRIs) to drive basic reproduction rate (R_0) to <1 is highly warranted.

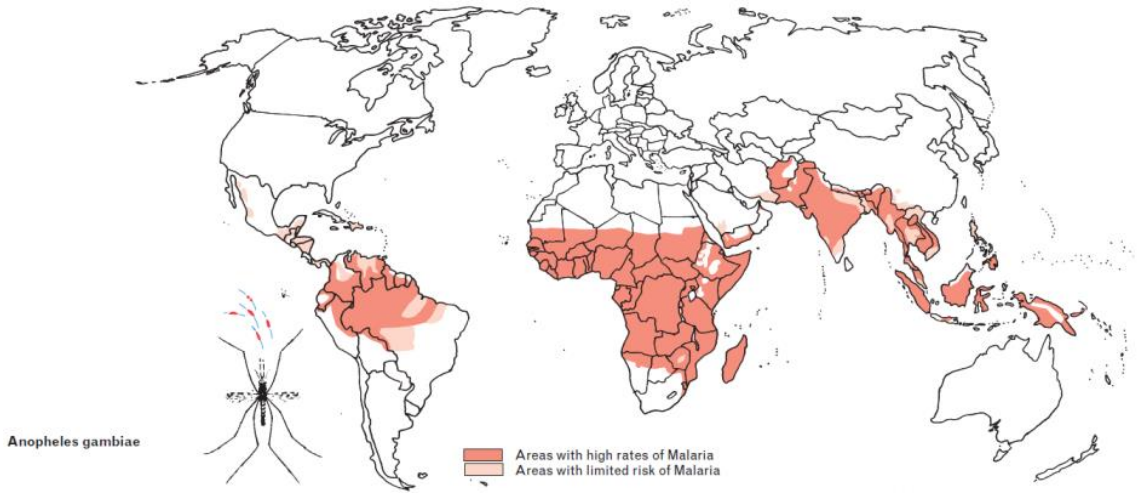


Figure 2.5: World malaria map showing disease transmission rates by *Anopheles gambiae* mosquitoes. Geographical distribution of transmission intensities across the tropics highlights a larger coverage of malaria burden in sub-Saharan Africa (Source: https://www.iamat.org/risks/malaria).

2.3 Malaria control strategies

2.3.1 Repellents and adulticides for mosquito control

To reduce human contacts with infectious bites of mosquitoes, the World Health Organization (WHO) recommends the use of long-lasting insecticide treated nets (LLINs) and indoor residual spaying (IRS) strategies that target in-house resting female mosquitoes. According to WHO statistics, expansive bed net ownership within endemic sub-Saharan African regions during 2014-2016 was estimated at 505 million for which 75% were distributed through mass distribution campaigns with coverage rising to 80% (http://www.who.int/malaria/media/world-malaria-report-2017/en/). The significant protection efficacy afforded by these barriers is contributed by the repellent and killing activities of the chemical ingredients of pyrethroids class. Synthetic pyrethroids used to impregnate bed nets and window curtains include; deltamethrin (**7**; Figure 2.6), permethrin (**8**), λ-cyhalothrin (**9**), cypermethrin (**10**), cyfluthrin (**11**), bifentrin (**12**) and etofenprox (**13**) (WHO, 1997).

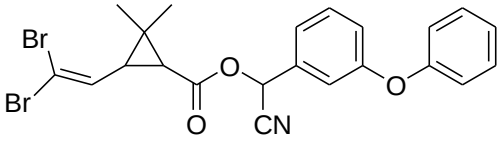
Intense campaigns by the WHO-supported Global Malaria Eradication Programme (GMEP; 1955-1969) to eradicate malaria during the World War II advocated for IRS that used dichlorodiphenyltrichloroethane (DDT) (**14**) to kill mosquito populations by several folds (Carter & Mendis, 2002). Several countries were declared malaria-free through this programme but unfortunately due to technical unsustainability of GMEP and widespread resistance to compound **14**; this led to collapse in vector containment efforts hence leaving malaria burden high in sub-Saharan Africa. Although compound **14** is still used in some west African countries after GMEP abandonment, other conventional insecticides spatially sprayed to kill adult mosquitoes include; organophosphates; malathion (**15**), fenitrothion (**16**), diazinon (**17**), organochlorides; dieldrin (**18**), hexachlorocyclohexane (HCH) (**19**). In consideration of human and environmental safety, the World Health Organization Pesticide Evaluation Scheme (WHOPES) approved the use of carbamates; pirimiphos-methyl (**20**), propoxur (**21**), bendiocarb (**22**), and pyrethroids; α-cypermethrin (**10**), permethrin (**8**), cyfluthrin (**11**), deltamethrin (**7**), bifenthrin (**12**), λ-cyhalothrin (**9**) and etofenprox (**13**) in IRS formulations (http://www.who.int/pq-vector-control/prequalified-lists/en/). In terms of functionality, pyrethroids and organochlorides irreversibly block voltage gate sodium channels (VGSC) of neurotransmission, while organophosphates and carbamates reversibly inactivate acetylcholinesterase (AChE), knocking down the insects and due to excessive synaptic excitation, the insects die.

Operational scale-up of IRS and LLINs interventions has resulted into reduced epidemiological malaria incidences, however, physiological and behavioral resistance has consequently developed, threatening control interventions and leaving humans barely unprotected (Ranson & Lissenden, 2016). In fact, a number of studies from endemic sub-Saharan Africa regions have reported loss of bed net effectiveness and high residual sporozoite inoculation rates as well as extensive resistance to IRS chemicals (Abraham, Massebo, & Lindtjørn, 2017; Mwesigwa et al., 2017; Riveron et al., 2018). Increased frequencies of insecticide resistance allelic point mutations in the vgsc (*Vgsc*-L1014S) and knock down resistance gene (*kdr*-L1014F) have been observed in *An.*

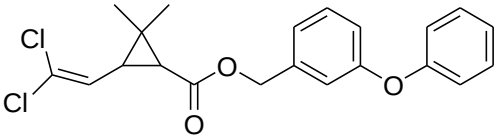
gambiae on exposure to pyrethroid compounds **7**, **8** (Kawada et al., 2014). Similarly, resistant mosquitoes carrying G119S mutation in *ace-1* gene successfully survive carbamates and organophosphate applications. Additionally, amplified gene copy numbers of cytochrome P450, esterases, and glutathione *S*-transferases involved in detoxification pathways have been widely associated with insecticide resistance and vector control failures. In his commentary summarizing US President's Malaria Initiative (PMI) funded-IRS in sub-Saharan Africa, Oxborough, (2016) suggested that sustainability of the programme urgently requires new insecticide portfolio following worrisome mosquito resistance to nearly all of the chemicals used, else a foreseen failure similar to GMEP ensues. Mosquitoes have consequently evolved adaptive survival mechanisms by biting outdoors and before bedtime, expanding host ranges to wild and domestic animals (Russell et al., 2011; Killeen, 2014; Cooke et al., 2015; Msellemu et al., 2016).

Repellent use has been proposed as a short-lived alternative to reduce outdoor mosquito bites by applying the formulations on the skin and garments (Debboun et al., 2006). Application of commercial repellent DEET (*N*,*N*-diethyl-m-toluamide)(**23**) offer ~99.8% protective efficacy against biting insects including mosquitoes. Other commonly used synthetic repellents include; dimethyl phthalate (**24**), *n*-propyl-*N*,*N*-diethyl-succinamate (**25**), 2-ethyl-2-butyl-1,3-propanediol (**26**), 2-ethyl-1,3-hexanediol (**27**), IR3535 (3-(N-acetyl-N-butyl) amino propionic acid ethyl ester) (**28**), picaridin (2-(2-hydroxyethyl)-1-methylpropylester) (**29**), and *O*-chloro-*N*,*N*-diethylbenzamide (**30**). In their investigations to decipher the mechanisms of protection, Bohbot & Dickens (2012) showed that repellents either mask human kairomones or bind onto odorant receptors blocking their elicitation therefore reducing human-vector contacts. Despite their guaranteed protection efficiency, synthetic repellents are costly, unaffordable to low-income residents living in malaria endemic regions and periodically induce allergic reactions. Imperatively, local residents tend to burn aromatic plants and use plant-based repellents to keep mosquitoes at bay (Maia & Moore, 2011; Muema et al., 2017). Among the most effective plant-derived repellents established so far are MR08 (menthol

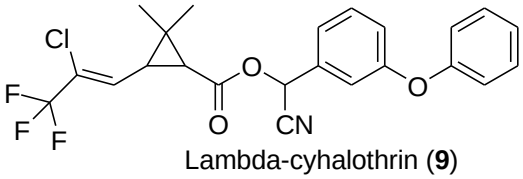
propylene glycol carbonate) (**31**) and *para*-methane 3,8-diol (PMD) (**32**) that has been approved for military and public use (Carroll & Loye, 2006; Kweka et al., 2012).



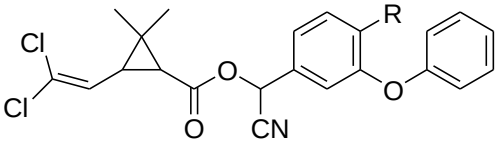
Deltamethrin (**7**)



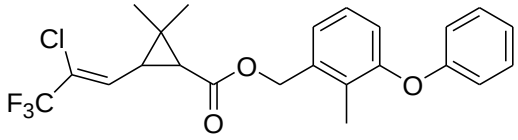
Permethrin (**8**)



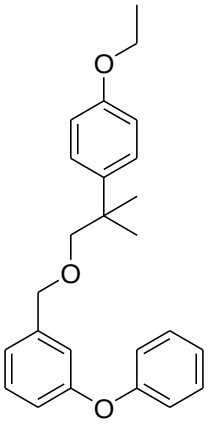
Lambda-cyhalothrin (**9**)



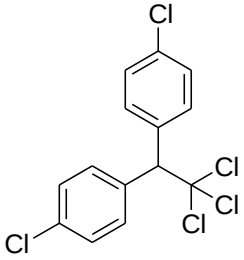
Cypermethrin (**10**) R = H
Cyfluthrin (**11**) R = F



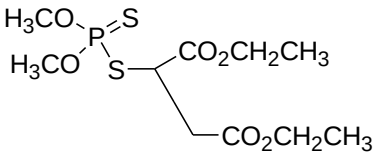
Bifentrin (**12**)



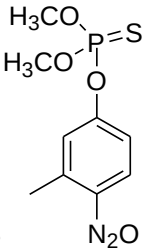
Etofenprox (**13**)



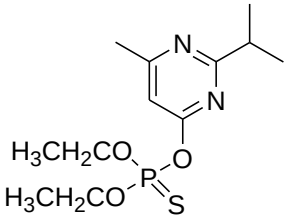
DDT (**14**)



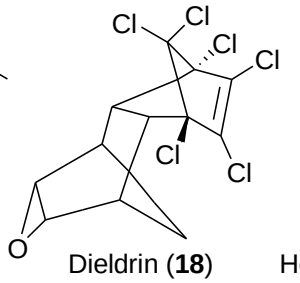
Malathion (**15**)



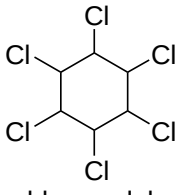
Fenitrothion (**16**)



Diazinon (**17**)



Dieldrin (**18**)



Hexachlorocyclohexane (**19**)

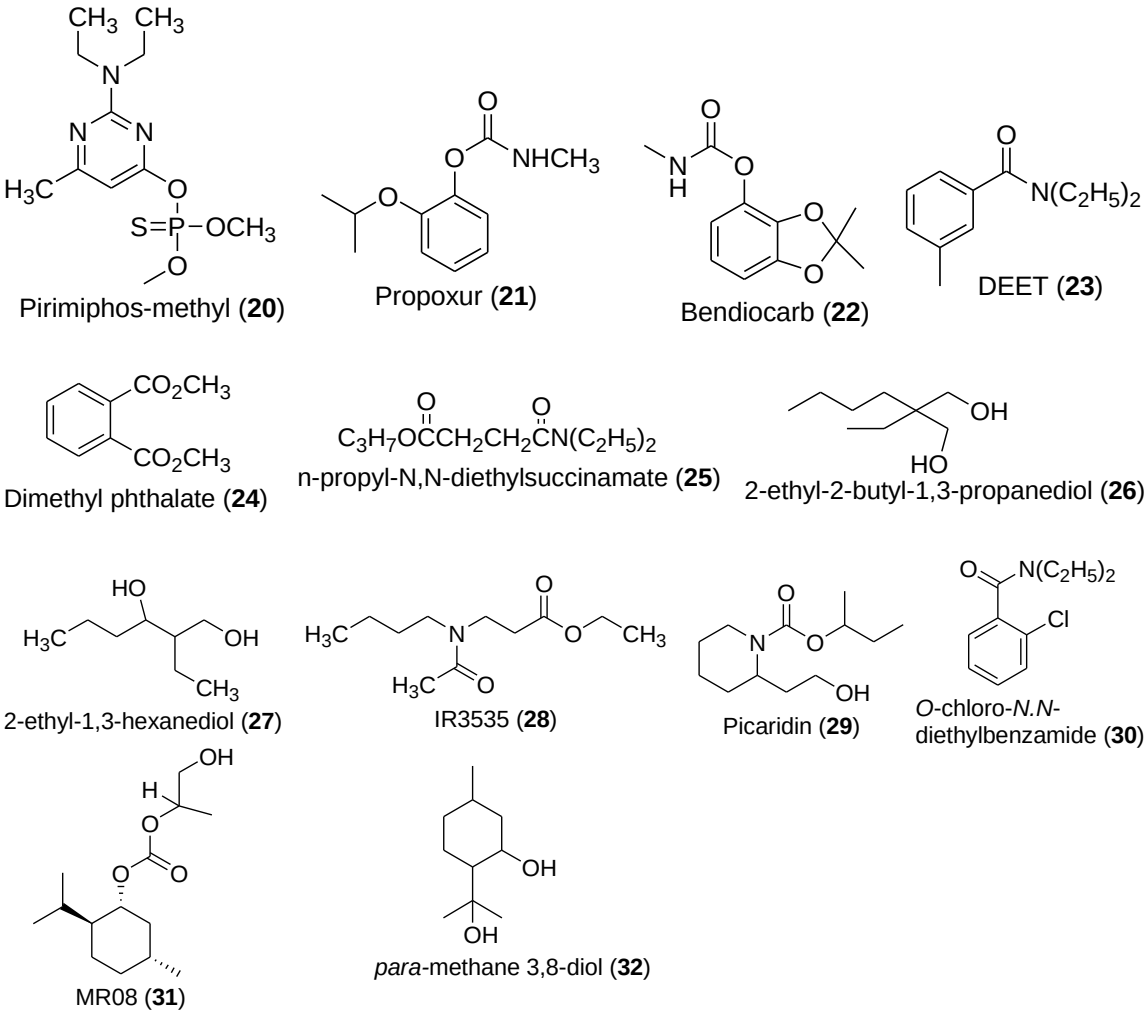


Figure 2.6: Mosquitocidal and repellent chemical compounds

2.3.2 Larviciding

As one of the historical interventions known to reduce adult mosquito populations by significant magnitudes, larviciding targets the less mobile and immature aquatic mosquito stages that are quite susceptible to chemical attacks and with less chances of developing resistance (Killeen et al., 2002a; Killeen et al., 2002b). Larviciding was first demonstrated to kill mosquito larvae using kerosene oil by Ronald Ross in Sierra Leone (1899) as a viable tool to reduce adult mosquito densities. However, this practice has been overlooked in current vector control programmes as many perceive it laborious and with varying degrees of success.

Back in 2009, larviciding programmes relied on the application of chemicals such as Malathion (**15**), DDT (**14**), bendiocarb (**22**), fenitrothion (**16**), chlorpyrifos (**33**; Figure 2.7), parathion (**34**), temephos (**35**), dieldrin (**18**) and propoxur (**21**) (WHO, 2009). However, increased insecticide resistance to most of these compounds, pollution of environment and gradual loss of biodiversity led to their phase out. Currently, the approved larviciding formulations constitute compounds, organochlorides; temephos (**35**), pirimiphos-methyl (**20**), insect growth regulators (IGRs); pyriproxyfen (**36**), diflubenzuron (**37**), fungal spinosyns; spinosad A and B, and bacterial spores from *Bacillus thuringensis* (Bti) and *B. sphaericus* (http://www.who.int/pq-vector-control/prequalified-lists/en/). Other chemical compounds that structurally mimic endogenous insect growth hormones are also used to control mosquito larvae, and include; methoprene (**38**), halofenozide (**39**), tebufenozide (**40**), fenoxycarb (**41**), methoxyfenozide (**42**), triterpenoid azadirachtin A (**43**) and neem chippings. Besides the application of chemicals, monosurface films and larvivorous fish offer alternative strategies to suppress mosquito populations to varied degrees, and are integratively used alongside other larvicides.

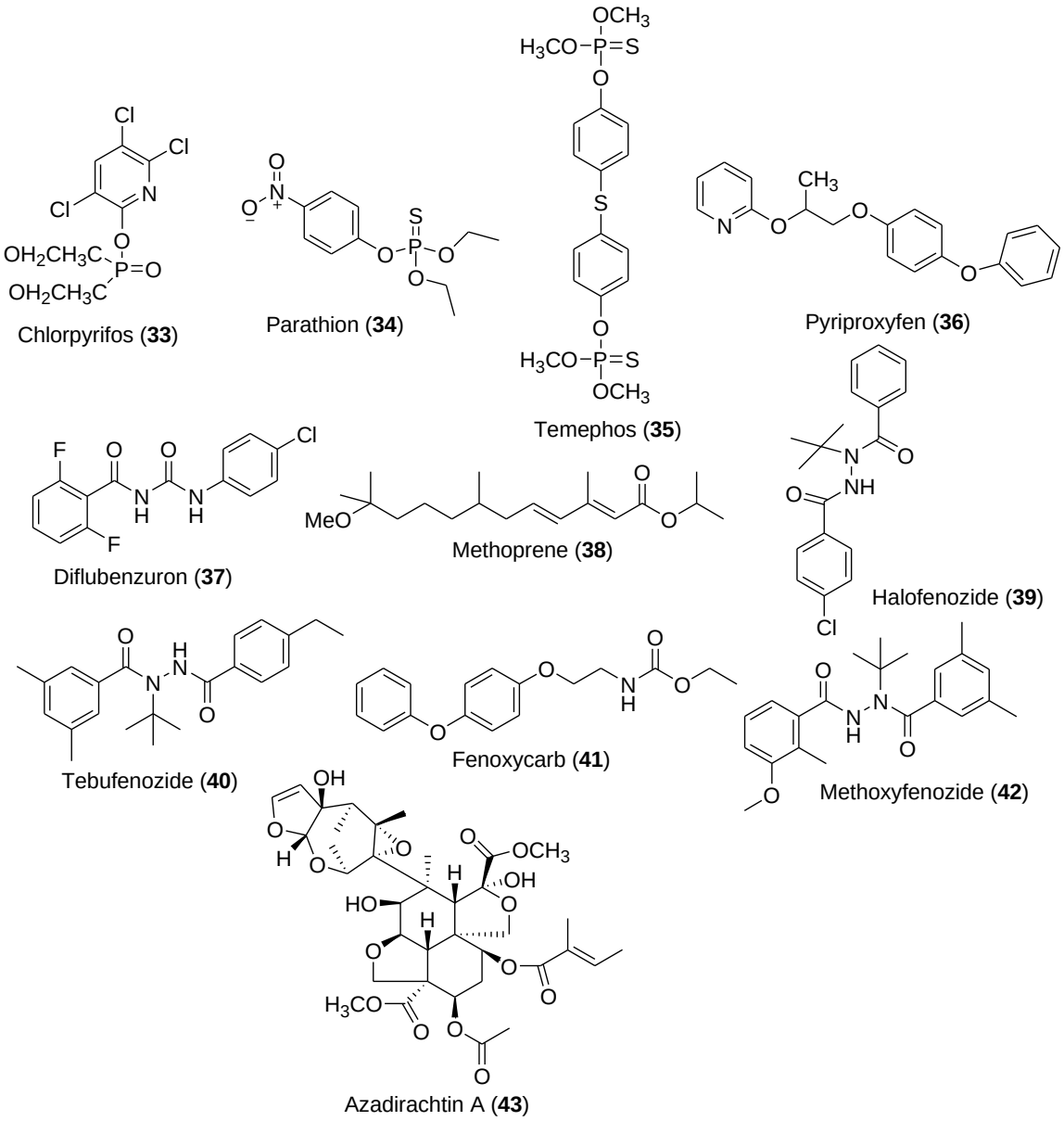


Figure 2.7: Larvicidal chemical compounds

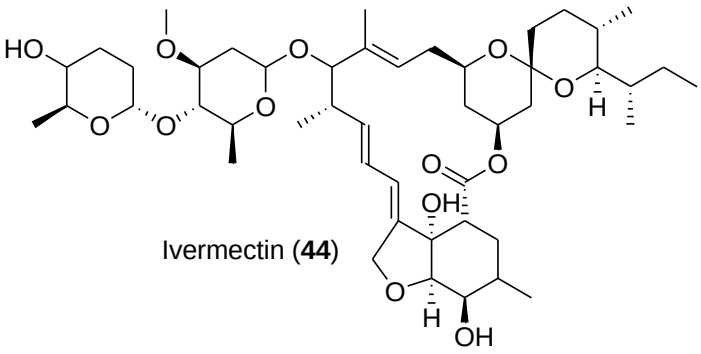
2.3.3 Use of endectocides in malaria control

Considered to drive elimination of malaria vectors, rather the intensified use of IRS and LLINs after the Abuja Declaration in 2000 have consequentially forced mosquitoes to behaviorally feed outdoor and to a larger extent on livestock, a characteristic feature maintaining residual transmissions (Reddy et al., 2011; Russell et al., 2011; Padonou et

al., 2012; Killeen, 2014; Meyers et al., 2016). Exploitation of this behavioral change towards a successful malaria control has been of great interest over the recent years in search for alternative vector control strategies (Foy et al., 2011; Chaccour et al., 2013; Russell et al., 2013; Chaccour & Killeen, 2016; Russell et al., 2016; Killeen et al., 2017; Waite et al., 2017). Macrocyclic lactones, especially ivermectin (**44**; Figure 2.8), which are historically proven to be potent antiparasitics in veterinary medicine (Campbell et al., 1983) have been shown to be repurposefully effective in vector control and disease transmission-blocking. Notably, the fact that effective treatment of onchocerciasis-infected cattle with ivermectin in Australian trials by Dr. Ian Hotson's group suggested blueprint extension to prevention of human river blindness whose approval was granted in 1987 (Campbell, 2016). Since then, the well tolerated and effective ivermectin (branded as Mectizan® at 150-200 µg/kg) has been reliable for mass drug administration (MDA) campaigns against onchocerciasis and microfilariasis in poor developing countries inflicted by these diseases (Cupp et al., 2011).

Contextually, to date, the systemic administration and/or topical application of ivermectin to cattle feasibly reduces the survival, feeding frequencies, blood digestability, locomotion activity and fecundity of blood sucking arthropod disease vectors including mosquitoes and tsetse flies (Kobylinski et al., 2010; Pooda et al., 2013; Pooda et al., 2015; Lyimo et al., 2017; Sampaio et al., 2017). As human malaria transmission greatly relies on the longevity of female mosquito, reduction of survivorship rates from this intervention breaks parasite transmission especially the outdoor feeding vectors. In fact, experimental studies to demonstrate the malaria transmission-blocking activities of ivermection have been widely explored at both laboratory and field levels. Findings from these studies show potential inhibitory activities against sporogonic and sporozoite development upon ingestion (Kobylinski et al., 2012; Kobylinski et al., 2017; Pinilla et al., 2018). Furthermore, findings from single-dose mass drug administration of ivermectin in western Africa regions as well as randomized clinical trials conducted in western Kenya suggest that the intervention could sustainably push impetus of malaria elimination (Sylla et al., 2010; Kobylinski et

al., 2011; Alout et al., 2014; Smit et al., 2018). Heavy concerns of possible development of cross resistance at field level have been raised. However, despite these concerns, mosquito vector control efforts have been extended to the development of ivermectin-based attractive toxic sugar baits against *An. arabiensis* (Tenywa et al., 2017). By using this prototype, over 95% mosquitoes were found knocked down 48 h post feeding on 10% sucrose solutions containing 0.01% ivermectin, suggesting its potential use for outdoor implementation.



Ivermectin (**44**)

Figure 2.8 Chemical structure of ivermectin (44).

2.3.4 Malaria vaccine development

The most awaited breakthrough in malaria control is the development of an effective vaccine that not only protects humans fully against disease severity but also breaks subsequent transmissions. However, the hard-to-answer question lingering into many minds is: how far or close are we from getting an effective malaria vaccine? (Sinden, 2010; Laurens, 2018). Despite the tribulations faced along the path of this venture since 1973, various promising vaccine candidates targeting parasite-specific surface proteins expressed at different stages of *Plasmodium* life cycle have been developed though displaying varying degrees of success in human protection (Jones & Hoffman, 1994; Crompton, Pierce, & Miller, 2010; Birkett, 2016). This compelling differential effectiveness arise from low immunogenicity and unexpected human genetic variations. A recombinant candidate vaccine, at an advanced level, RTS,S (Mosquirix™) unveiled by a partnership between GlaxoSmithKline (GSK) with the PATH Malaria Vaccine Initiative (MVI), comprising of 25% sporozoite surface subunits CSP and TRAP linked to AS01 adjuvant, and 75% wild-type hepatitis B antigen (HBsAg) entered clinical trials ("A Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Infants," 2012). The first candidate vaccine trials have been conducted in malaria-vulnerable infants where ~ 15,000 African children in 11 centre sites were recruited for efficacy assessment from 2009 through November 2014. This second generation vaccine candidate only targets the pre-erythrocytic malaria infections by inhibiting sporozoite motility and preventing hepatocyte invasions with a well tolerability profile in humans (Arama & Troye-Blomberg, 2014; Rts, 2014). The protection is thought to occur through induction of anti-CSP antibodies inhibiting sporozoite-mediated cell transversal and establishment of infection (Mishra et al., 2012).

In 2015, phase III clinical trials of RTS,S/AS01 implemented in seven (7) African countries; Kenya, Gabon, Burkina Faso, Ghana, Tanzania, Mozambique, and Malawi registered a four-dose efficacy of as low as 27% and 30-50% protection in vulnerable infants (6-12 weeks old) and adults, respectively (RTS, 2015). Amazingly, despite its short-lived immune boost and low success rates, the European Medicines Agency was

impressed by these outcomes and further recommended its large-scale country-level late-stage phase III implementations that remained subject to WHO approval. In 2018, following recommendations during the Malaria Vaccine Symposium at Baltimore, USA, WHO announced commencement of Phase IV RTS,S pilot implementations in Ghana, Kenya, and Malawi giving hope for future advancements towards achieving an effective malaria control (Coelho et al., 2017). In October 2021, the WHO recommended its broad use in children within high malaria endemicity. Besides this great milestone, with the evidential low clinical field efficacy and dwindling uncertainty of delivering a fully protective malaria vaccine against various parasite strains, the remaining hope rekindles in antimalarial drug discovery.

2.3.5 Antimalarial chemotherapy and drug resistance status

Before drug molecules came into existence, earlier written records highlight that humankind was faced with feverish illnesses for which treatment and prophylactic measures involved the use of plant extracts. Not surprisingly that malaria-like fever developed by early Jesuit missionaries in Peru during the 17th Century was cured by then unidentified "contaminated tonic water" compounds from indigenous *Cinchona succuriba* plants (Meshnick & Dobson, n.d.). Later in 1820, French chemists Pierre Pelletier and Joseph Caventou made the first major breakthrough in antimalarial therapy by successfully isolating quinoline alkaloid quinine (QN) (**45**; Figure 2.9) from cinchona barks (Wells, 2011). The wonder drug molecule **45** has since then been effective therapy for severe malaria in children albeit its toxicity side effects. Quinine (**45**) provided a blueprint for the synthetic development of 4-aminoquinoline quinine analogue chloroquine (CQ) (**46**) during the World War II, increasing safety and potency (Stork et al., 2001). Although compound **46** had not yet gained popularity after its 1934 manufacture by the German scientists, its superb antimalarial effectiveness was overwhelmingly known in 1950s during the first attempts of GMEP (Wongsrichanalai et al., 2002). The precise mode of action of **45** and **46** remains unknown, but using X-ray crystallography the two compounds are highly postulated to accumulate within the parasite's digestive food vacuole inhibiting hemozoin formation (Table 2.1). By

attaching to heme derivatives (ferriprotoporphyrin IX, FPIX), compounds **45**, **46** and other quinoline derivatives inhibit FPIX biocrystallization resulting into creation of highly oxidative and toxic environment for the asexual blood stages (de Villiers & Egan, 2009). Survival of *Plasmodium* against these compounds has been attributed to point mutations K76T and N86Y on membrane-associated drug transporter *Pf*CRT as well as overexpression of *Pf*MDR1 and polymorphisms on *Pf*nhe-1 that mediate resistance (Wongsrichanalai et al., 2002). Emergence and vast spread of CQ-resistant *P. falciparum* parasites first reported in South East Asia and Latin America in the late 1960s to endemic African region (first reported in 1978) through global hard selective sweeps led to its withdrawal as first line treatment (Packard, 2014). CQ is used even today for uncomplicated malaria treatment despite its declined efficacy against drug resistant strains. Amazingly, a number of studies show that the withdrawal of CQ has reversed the parasites' sensitivity from the selective pressure of the drug in Malawi, Ethiopia, Zambia, and Kenya (Laufer et al., 2010; Frosch et al., 2014; Mekonnen et al., 2014; Kiarie et al., 2015; Mwanza et al., 2016).

Scientific advances during the World War II resulted in development of quinoline analogs including mefloquine (**47**), amodiaquine (**48**), halofantrine (**49**), lumefantrine (**50**), primaquine (**51**), tafenoquine (**52**), pyronaridine (**53**) and piperaquine (**54**) by Americans between 1950-1975 to avert the thwarting CQ resistance (Wells et al., 2009). Resistance to most of these compounds started being reported in Thailand-Cambodia border in 1975, late 1980s, late 1990, (Schlitzer, 2007). Using the mouse model parasite in *P. berghei* ANKA, Kiboi et al., (2014) associated *Plasmodium* resistance to **50** and **54** with increased expression of *Pb*vp2 and *Pb*cvx1. Recently, Wong et al. (2017) demonstrated that mefloquine (**47**) efficacy is afforded by the targeted inhibition of protein synthesis.

Following the widespread failure and unsustainability of CQ and its relative quinolones to combat malaria in many countries, the dual-acting sulfadoxine (**55**)-pyrimethamine (**56**) (SP) (Fansidar®) that target folate biosynthesis pathway was introduced late 1960s

(Eriksen et al., 2008). Unfortunately, the effectiveness of SP did not last long with antimalarial resistance associated with mutations on dihydrofolate reductase (dhfr) was reported in 1967 in Thailand and late 1980s in Africa (Wongsrichanalai et al., 2002). Changes in codons S108N, C59R, N51N, and I164L of dhfr were associated with pyrimethamine resistance, while mutations in dihydropteroate synthase (dhps) confer resistance to sulfadoxine (Inoue et al., 2014). Other antifolate compounds used as antimalarials are proguanil (**57**), dapsone (**58**), and chlorcycloguanil (**59**). Later in 2000, a hydroxynapthoquinone derivative atovaquone (ATQ) (**60**) known to inhibit mitochondrial respiratory chain (Fry & Pudeny, 1992) in fixed combination with proguanil (**57**) (trade marked as Malarone®) became the preferred antimalarial therapy following **60** resistance as monotherapy (Marra et al., 2003). Resistance to Malarone® in some regions of West Africa and Thailand has been reported (Kuhn et al., 2005).

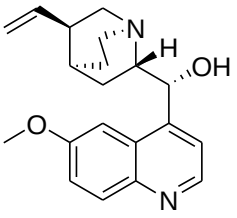
In 1972, Tu Youyou and co-workers discovered a sesquiterpene lactone Artemisinin (**61**) from a Chinese weed Qing hao (*Artemisia annua*) that the world appreciated its wonderful antimalarial efficacy to-date by awarding her a Nobel Prize in 2015. While this does not mean that the extract was not used as antimalarial therapy in China, the plant was in use for ~ 2000 years, but little was known of the active ingredients before the discovery (Meshnick & Dobson, n.d.). Compound **61** has poor bioavailability due to its insolubility in water and oil, thus efforts to synthesize the molecule chemically in 1990 led to a number of its derivatives including dihydroartemisinin (DHA) (**62**), Arteether (**63**), artemether (**64**), artesunate (**65**), and artelinic acid (**66**) (Meshnick, 2002). The mechanism of action of artemisinins has not been fully resolved yet but, studies postulate that these compounds kill *Plasmodium* parasites by damaging their proteins and inhibiting proteasome (Bridgford et al., 2018). Elsewhere, Taylor et al., (2004) proposed that the endoperoxide bridge in artemisinin derivatives interacts with heme in the digestive vacuole generating toxic free radicals and disruption of mitochondrial membrane potential. Another study by Eckstein-Ludwig et al., (2003) strongly associates the antimalarial activity of artemisinins with inhibition of *Plasmodium* sarcoendoplasmic reticulum Ca^{2+} (SERCA) ATPase (*Pf*ATP6).

The escalating antimalarial drug resistance and fear to lose the fight against the deadly disease led to WHO's recommendation of the adoption of artemisinin combination therapy (ACT) as first line treatment regimen for uncomplicated malaria in 2005, a concept derived from antimicrobial treatment (Maude et al., 2010). Partner drugs in ACT include mefloquine (**47**), lumefantrine (**50**), pyronaridine (**53**), and piperaquine (**54**). Deployment of this ACT strategy in combination with intensified vector control interventions remarkably reduced malaria transmission intensities until 2010 when treatment failure started to be reported and ultimate stall in malaria control reported in 2017 (Dondorp et al., 2009; Na-Bangchang et al., 2013; Saunders, Vanachayangkul, & Lon, 2014; Dondorp et al., 2017).

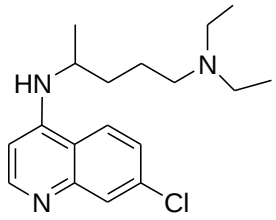
First reports of ACT resistance emerged from South East Asian (SEA) countries in early 2010 (Figure 2.10) with *Plasmodium* parasites exhibiting phenotypic characteristics; slow clearance rates, high recrudescence, quiescence, delayed death, increased *Pf*P13K levels associated with C580Y mutation and enhanced unfolded protein response (Table 2.1) (Dondrop et al., 2009; Cheng, Kyle, & Gatton, 2012; Mbengue et al., 2015; Shaw et al., 2015). Additionally, the ACT resistance is associated with parasites carrying point mutations G533A, R539T, I543T and C580Y on propeller *Kelch13* gene locus (Ariey et al., 2014; Ashley et al., 2014; Talundzic et al., 2015; Fairhurst & Dondorp, 2016; Blasco, Leroy, & Fidock, 2017). Currently, molecular surveillance studies indicate that artemisinin resistant parasites emerged independently, and widely spread in SEA contributing to massive ACT failure (Huang et al., 2015; Takala-Harrison et al., 2015), a scenario depicting possible soft selective sweep evolutionary forces on the major chromosome 13 (Miotto et al., 2013; MalariaGEN Project, 2016). Although the hardest hit continent by malaria, sub-Saharan Africa, has not experienced high epidemiological prevalence of *Pf*Kelch13 mutations reported in SEA (Taylor et al., 2015), low allelic frequencies of non-selected unique non-synonymous mutations A578S and R622I were recently reported (Kamau et al., 2015; Bayih et al., 2016). Owing to the targeted elimination mission set out by many countries, great fear of ACT resistance spreading

throughout Africa alongside the widespread insecticide resistance raises the alarm to the challenging future of combating malaria in this endemic region.

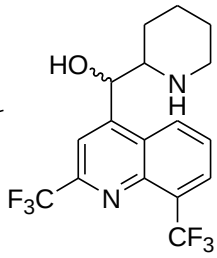
Antibiotics such as doxycycline (**67**), clindamycin (**68**), and azithromycin (**69**) also serve as antimalarials by targeting the parasite's apicoplast fatty acid biosynthesis. Due to their slow action, these antibiotics are used in combination with fast acting antimalarial drugs such as quinine (**45**) and artesunate (**65**).



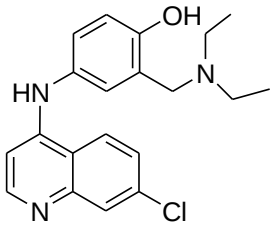
Quinine (**45**)



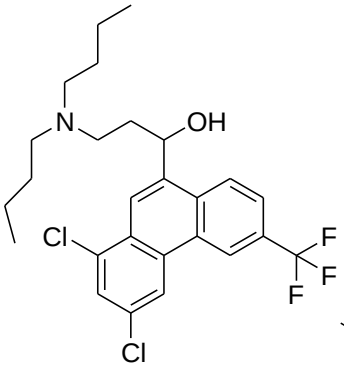
Chloroquine (**46**)



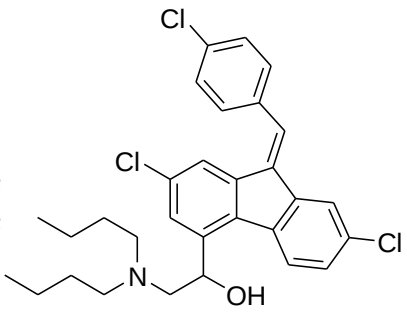
Mefloquine (**47**)



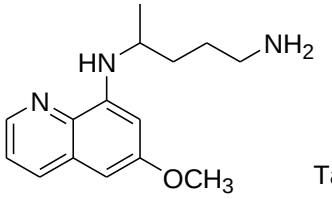
Amodiaquine (**48**)



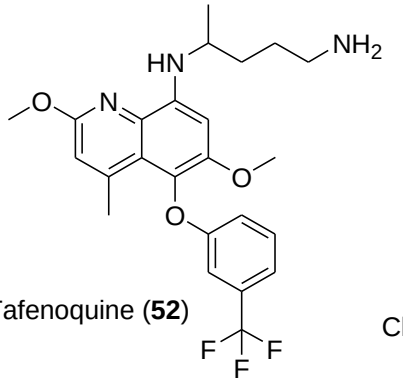
Halofantrine (**49**)



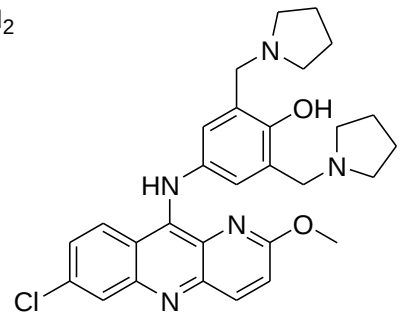
Lumefantrine (**50**)



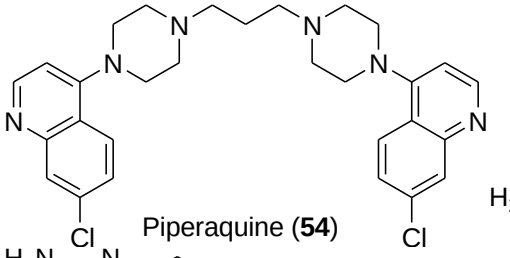
Primaquine (**51**)



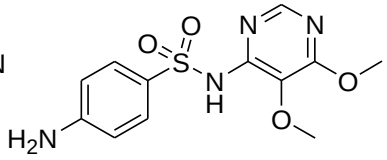
Tafenoquine (**52**)



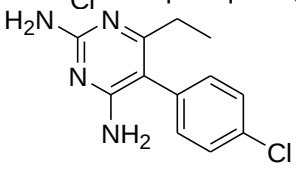
Pyronaridine (**53**)



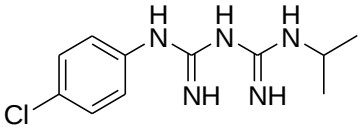
Piperaquine (**54**)



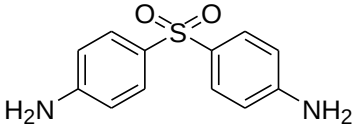
Sulfadoxine (**55**)



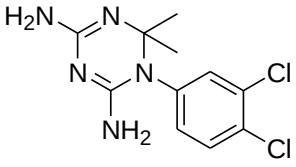
Pyrimethamine (**56**)



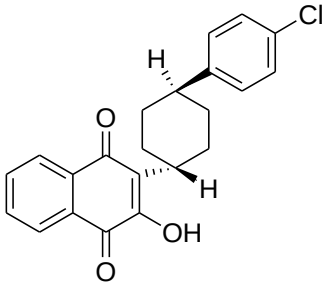
Proguanil (**57**)



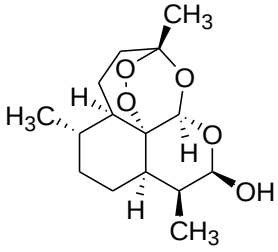
Dapsone (**58**)



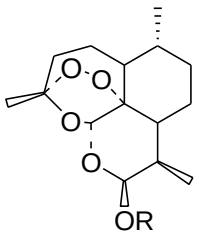
Chlorcycloguanil (**59**)



Atovaquone (**60**)



Artemisinin (**61**)



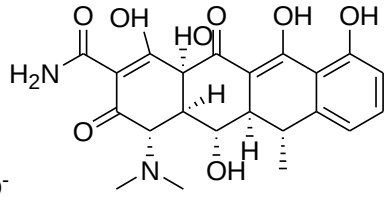
Dihydroartemisinin (**62**) R=H

Arteether (**63**) R=Et

Artemether (**64**) R=Me

Artesunate (**65**) R= $(OC)CH_2CH_2COO^-$

Artelinic acid (**66**) $R=CH_2Ph(COOH)$-P



Doxycycline (**67**)

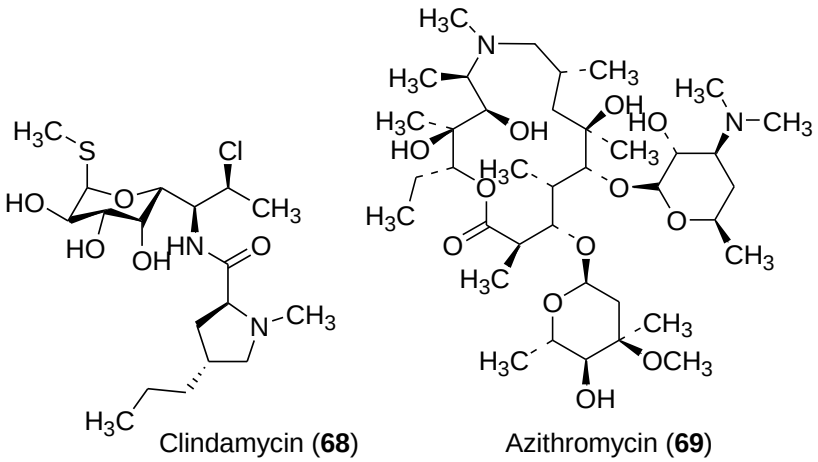


Figure 2.9: Chemical structures of antimalarial drugs.

Table 2.1: Antimalarial drugs, their mechanisms of action and resistance mutations

Chemical class	Antimalarial drugs	Mechanism of action	Resistance proteins and mutations	References
Artemisinin and its derivatives	Artemisinin (**61**), Dihydroartemisinin (DHA) (**62**), Arteether (**63**), Artemether (**64**), Artesunate (**65**)	SERCA ATPase**,** damaging proteins and ubiquitin-proteosome inhibition	*Pf*Kelch 13	Bridgford et al., 2018; Dogovski et al., 2015; Eckstein-Ludwig et al., 2003
Antifolates	Sulfadoxine/Pyrimethamine (**55/56**), Dapsone (**58**), Proguanil (**59**)	Block folate biosynthesis	Point mutations on *Pf*dhfr, *Pf*dhsp	Inoue et al., 2014
Aryl amino alcohols	Quinine (**45**), Mefloquine (**47**), Halofantrine (**49**), Lumefantrine (**50**)	Inhibit heme detoxification ; Mefloquine inhibit protein synthesis	*Pf*CRT (K76T), PfMDR1 (N86Y)	Schlitzer, 2007; Wong et al., 2017
8-aminoquinolines	Primaquine (**51**)	Kills stage V gametocytes	-	Lelièvre et al., 2012
4-aminoquinolines	Chloroquine (CQ) (**46**), Amodiaquine (AQ) (**48**)	Inhibition of hemozoin formation	*Pf*CRT (K76T), PfMDR1 (N86Y)	Wongsrichanalai et al., 2002
Respiratory chain inhibitors	Atovaquone (**60**)	Targets mitochondrial respiratory chain	Cytochrome b C1	Kuhn, Gill, & Kain, 2005; Naoshima-Ishibashi et al., 2007
Antibiotics	Doxycycline (**67**), Clindamycin (**68**), Azithromycin (**69**)	Inhibit mitochondrial and/or apicoplast metabolic processes	-	Dahl & Rosenthal, 2007

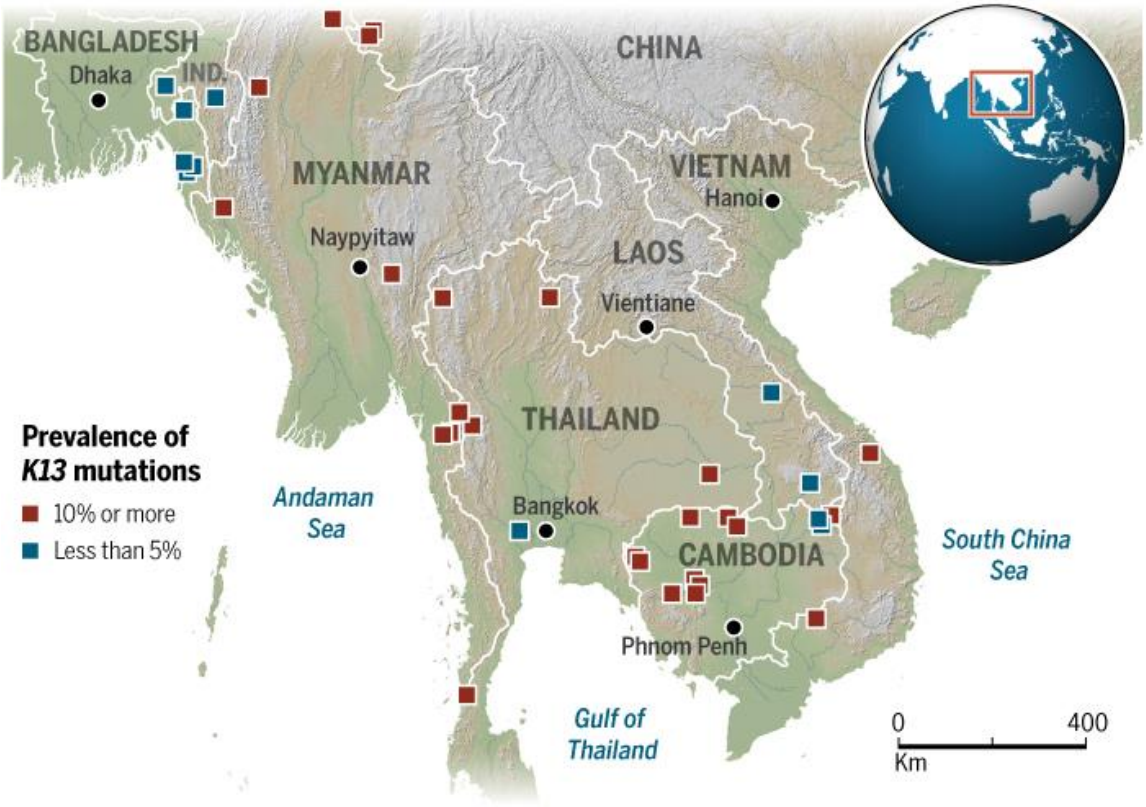


Figure 2.10: Distribution of Artemisinin resistant *P. falciparum* parasites with K13 mutations in South East Asian countries.

2.3.5.1 Current hits in drug development pipeline and clinical trials

Major public-private partnerships; GlaxoSmithKline, the Medicines for Malaria Venture (MMV), and Bill & Melinda Gates Foundation formed over the recent years have collaboratively embarked on supporting the development of next-generation antimalarials through fostering of medicinal chemistry campaigns as well as novel high-throughput drug screens (Diagana, 2015; Burrows et al., 2017). Since 1996 no new drug molecule had been introduced into clinical pipeline, and through this platform a number of open malaria box libraries comprising of thousands of chemical compounds have been established. Of such, collaborations between MMV, pharmaceutical companies, and several academic researchers across the globe have identified novel lead antimalarial compounds currently at different stages of clinical trials (http://www.mmv.org/research-

development/interactive-rd-portfolio; Held et al., 2014; Bhagavathula, Elnour, & Shehab, 2016; Table 2.2).

Through high-throughput whole cell (phenotypic) screening using asexual blood stages of *P. falciparum*, Rottmann and co-workers discovered Spiroindolone KAE609 (cipargamin, NITD609) (**70**; Figure 2.11) from ~12,000 natural products at Swiss Tropical and Public Health and synthesized by Novartis (Rottmann et al., 2010). As a Na^+ *Pf*ATP4 inhibitor with 7-times potency than Artesunate (**65**) and 40-times more than 4-aminoquinolines, NIT609 displayed remarkable Phase I results and currently at Phase II trials. Compound **70** exerts its activity on parasites at schizont stage (schizonticidal) and active against artemisinin-resistant mutants as well as gametocytes (van Pelt-Koops et al., 2012; Spillman et al., 2013). Another candidate molecule identified through phenotypic screen is an imidazolopiperazine KAF156 (formerly known as GNF156) (**71**) that is less potent than **70**. Compound **71** acts at liver stages (hypnozoites) thus providing prophylactic protection against *P. falciparum* and *P. vivax* at low nanomolar doses. Furthermore, the molecule has demonstrated possible gametocytocidal activity blocking mosquito transmissions and currently a candidate under Phase II clinical trials (Flannery, Chatterjee, & Winzeler, 2013). A dihydroorotate dehydrogenase (DHODH) inhibitor DSM265 (NCT02123290) (**72**) is yet another candidate molecule identified through phenotypic screening. Compound **72** exerts activity against liver stages of *P. falciparum* and *P. vivax* at a single dose of 400 mg and Phase II clinical trials in Peru gave promising findings (Phillips et al., 2015). At the University of Dundee, UK, through similar phenotypic screening approach, a 2,6-di-substituted quinolone-4-carboxamide scaffold DDD107498 (**73**) was identified and developed from ~ 4,700 natural products (Baragana et al., 2015). Compound **73** targets liver stages, but also effective against various drug resistant *Plasmodium* strains.

Intensified medicinal chemistry campaigns have led to synthesis of a number of arsenals. Through a collaborative partnership between MMV, Nebarska University (USA), and Monash University (Australia), synthetic ozonides possessing endoperoxide bridge like

Artemisinin (**61**); OZ277 (1,2,4-trioxolane) (**74**) and OZ439 (**75**) have been developed (Vennerstrom et al., 2004; Charman et al., 2011). These compounds are believed to liberate free radicals upon reduction of the endoperoxide bridge by heme and ferrous iron produced upon heme degradation killing the parasite. Initially, compound **74** displayed promising results after completely curing *P. berghei*-infected mice at three 10 mg/kg oral doses, but its development was deprioritized following unstable efficacy during Phase II clinical trials (Olliaro & Wells, 2009). Consequently, a fast-acting and potent compound **75** (mesylate salt) was developed to replace the unstable **74**. A single dose of compound **75** at 30 mg/kg cures *P. berghei* infections and blocks transmission to mosquitoes (Charman et al., 2011). Currently, compound **75** is under Phase II trials after exhibiting promising Phase I results.

Other compounds under clinical trials have been developed from existing chemical scaffolds through structural modifications aimed at reducing toxicity, averting resistance and improving metabolic stability. An organometallic compound Ferroquine (SSR97193) (**76**), CQ analogue AQ-13 (R047-0543) (**77**) and SAR116242 (Trioxaquine) (**78**) all at Phase II trials were developed from either CQ or AQ (O'Neill et al., 2012). Being the first organometallic antimalarial analogue of CQ developed by Sanofi-aventis to enter clinical trials, **76** is thought to interact with lipids, inhibit hemozoin formation and induce liberation of toxic free radicals affording its antimalarial activity (Dubar et al., 2008). Displaying quite promising results during clinical trials (McCarthy et al., 2016), plans to combine compound **76** with an endoperoxide OZ439 (**75**) are under consideration for efficacy improvement. A 2-carbon side chain substituted CQ, **77** developed under a collaboration between Tulane University and Louisiana University (De et al., 1996) showed Phase I pharmacokinetic profile similar to CQ. While good progress of this antimalarial agent was observed, its continued evaluation to Phase II trials in Mali has been halted following adverse electrocardiac changes associated with similar aminoquinolines (Bhagavathula et al., 2016). Trioxaquine (**78**) developed by Meunier and co-workers was found active against CQ and **56**-resistant strains (IC_{50} 10 nM) possibly by alkylating heme and inhibiting hemozoin formation. Efforts to reduce

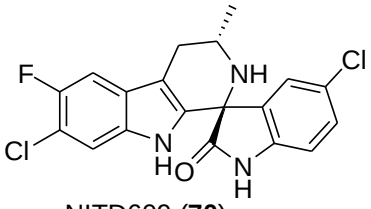
toxicity effects of primaquine (PQ, **51**) on G6PD patients have led to a 3-phenoxy-substituted 8-aminoquinoline tafenoquine (TQ; WR 238605) (**52**). TQ that is 4-100 times potent than **51** (Peters et al., 1993) has passed Phase II and III stages as a prophylactic agent against *P. falciparum* and *P. vivax* parasites. With such efficacy against gametocytes, hypnozoites, and liver stages, hemolytic concerns still raises alarm over its safety (Marcsisin et al., 2014). Other candidate compounds developed from existing drug scaffolds are Artemisone (BAY44-9585, **79**) and Sevuparin (DFOZ, DF02) (**80**), derived from Artemisinin (**61**) and heparin, respectively.

Deployment of target-based approach led to identification of compounds; methylene blue (**81**), and fosmidomycin (**82**) as potential antimalarials. More than a century ago, a Germany scientist Paul Ehrlich discovered a thiazine dye that could kill malaria parasites. The ability of **81** to kill gametocytes *in vitro* (Adjalley et al., 2011; Wadi et al., 2018) and reduce gametocytemia from 100 to 36% when co-administered with either artesunate (**65**) at a fixed dose 15 mg/kg has warranted it Phase I trials for transmission blocking (NCT01668433) (Held et al., 2015). In Phase II trials conducted in Burkina Faso, a monotherapy combination of methylene blue (**81**) with CQ proved safety in children against uncomplicated *P. falciparum* malaria (Meissner et al., 2006; Coulibaly et al., 2009). Fosmidomycin (**82**) is an antibiotic isolated from *Streptomyces lavendulae* that targets isoprenoid biosynthetic pathway in *Plasmodium* by inhibiting methylerythritol phosphate cytidyltransferase (IspD) and deoxyxylulose phosphate reductoisomerase (DXR) (Zhang et al., 2011). When administered alongside clindamycin (**68**), the antibiotics showed effective protection against uncomplicated malaria in adults and older children but poor efficacy in 1-2 years old children possibly due to poor immunity (Ruangweerayut et al., 2008). In efforts to find a better partner drug for fosmidomycin (**82**), Phase II trials are also focused on administration of compound **82** with piperaquine (**54**).

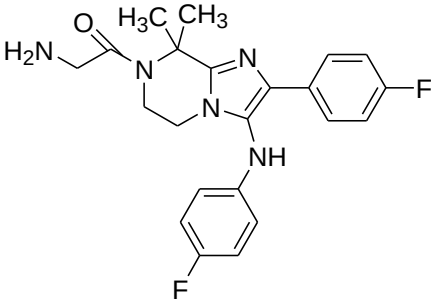
Table 2.2: Some antimalarials under clinical trials targeting different parasite life stages

Parasite target	Antimalarials	Phase of clinical trial	References
*i*RBC	Azithromycin (**69**) + Chloroquine (**46**)	III	MMV
	Ferroquine (**76**) +Artesunate (**65**)	II	Clinicaltrials.gov
	Fosmidomycin (**82**)+Clindamycin (**68**)	II	Clinicaltrials.gov
	Ozonide-OZ439 (**75**)	II	Charman et al., 2011
	Piperaquine (**54**)	IV	Warhurst et al., 2007
	Spiroindolone-NITD609 (**70**)	I	Rottmann et al., 2010
*i*RBC + Gametocytes	Artesunate (**65**)-Amodiaquine (**48**)	IV	MMV
	Artesunate (**65**) (i.v)	IV	MMV
	Coartem®(Artemether+Lumefantrine)	IV	MMV
	Eurartesim®(dihydroartemisinin+piperaquine)	IV	MMV
	Pyramax pediatric® (pyronaridine +artesunate)	III	MMV
*i*RBC+GM+LS	Imidazolopiperazine	I	Wu et al., 2011
	Timidazole	II	Clinicaltrials.gov
LS	Tafenoquine (**52**)	III	MMV
	Bulaquine	III	Valecha et al., 2001

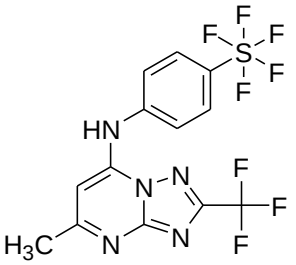
(Modified from Olliaro & Wells, 2009; Aguinar et al 2012)



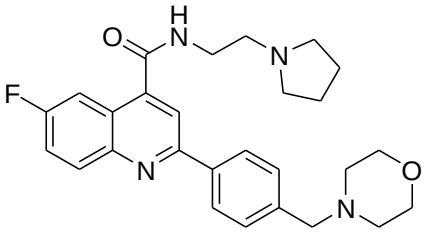
NITD609 (**70**)



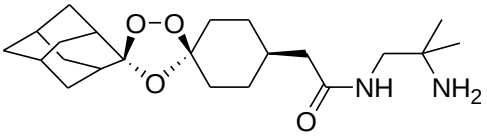
KAF156 (**71**)



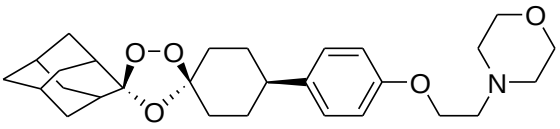
DSM265 (**72**)



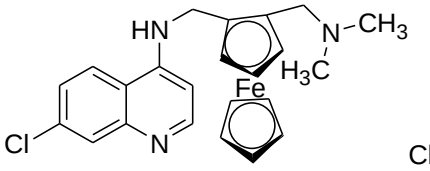
DDD107498 (**73**)



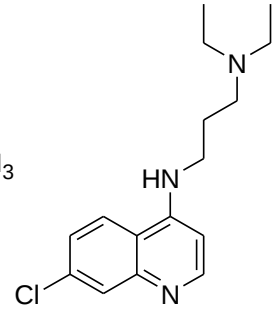
OZ277 (**74**)



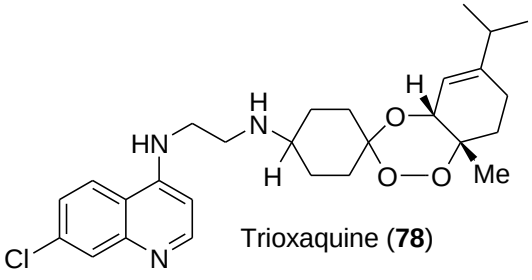
OZ439 (**75**)



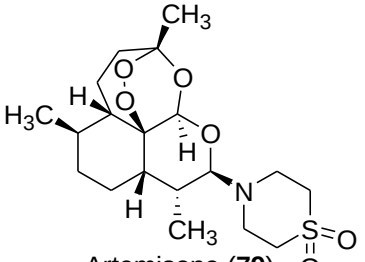
Ferroquine (**76**)



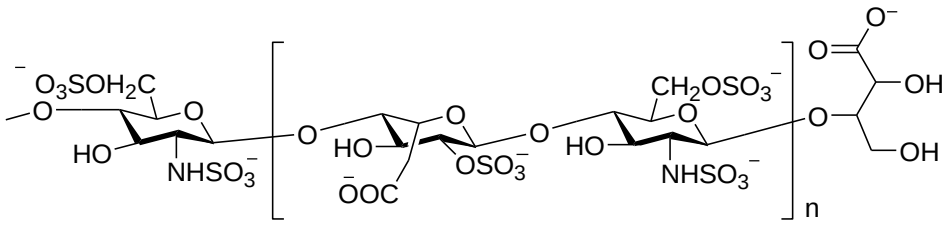
AQ-13 (**77**)



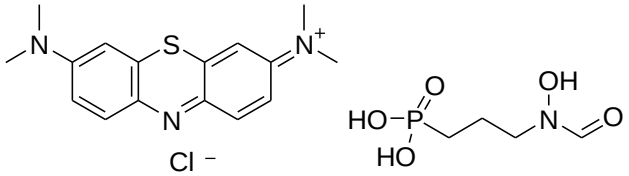
Trioxaquine (**78**)



Artemisone (**79**)



Sevuparin (**80**)



Methylene blue (**81**) Fosmidomycin (**82**)

Figure 2.11: Chemical structures of antimalarials under clinical trials pipeline

2.4 Naturally occurring plant-derived compounds as antimalarial agents and lead hits in antimalarial drug discovery

Antimalarial drug discovery from plants dates back since the isolation of quinine from Peruvian cinchona barks as a potent antimalarial agent by French chemists in 1820, highlighting plants as resourceful sources of effective anti-infectives (Wells, 2011), (Ginsburg & Deharo, 2011). In fact, some countries have licensed the use of plant extracts as antimalarial remedies. For instance, Brazil through the ministry of health approved the use of *Bidens pilosa* extract containing flavonoids and polyacetenes for human use (Aguiar et al., 2012). In Nigeria, China, and Ghana, several polyherbal products are quite often used for curative and prophylactic purposes. Potent plant-derived antimalarial compounds, including lapachol, artemisinin, curcumin, cryptolepine and the newly identified scaffold spiroindolone NITD609 are currently the classical prototypes for development of safer and effective antimalarial agents. From December 2000 to present, several reviews have compiled antimalarial activities of plant-derived extracts and their active principles (Schwikkard & van Heerden, 2002; Saxena et al., 2003; Frederich et al., 2008; Pillay, Maharaj, & Smith, 2008; Batista, De Jesus Silva Júnior, & De Oliveira, 2009; Bero, Frédérich, & Quetin-Leclercq, 2009; Kaur et al., 2009; Mariath et al., 2009; Adebayo & Krettli, 2011; Pohlit et al., 2013). Applying the activity cut-off limits of antimalarial compounds used by Willcox *et al*., (2004) and

Mahmoudi *et al*., (2006), most of the reported derivatives lie within the "good activity" (IC_{50} 1 - 20 µM) and "moderate activity" (IC_{50} 20 - 100 µM) limits. Only the promising antimalarial plant compounds with potent activities of $IC_{50} \leq 1.0$ µM (Figure 2.12 – 2.16) are discussed below.

2.4.1 Antimalarial alkaloids

Antimalarial alkaloids are among the most promising and versatile agents isolated from a number of plants around the globe (Figure 2.12). For instance, potent indolizidines prosopilosidine (**83**), isoprosopilosidine (**84**), prosopilosine (**85**), isoprosopilosine (**86**), and juliprosopine (**87**) have been isolated from the leaves of *Prosopis grandulosa* (Fabaceae), a closely related species to *P. juliflora* (SW.) DC. Antimalarial evaluation on these compounds demonstrated high activities against CQ-sensitive (D6) and CQ-resistant (W2) *P. falciparum* strains; prosopilosidine (**83**) and isoprosopilosidine (**84**) (IC_{50} 0.1 µM against D6 and 0.3 µM against W2), prosopilosine (**85**) (IC_{50} 0.19 µM against D6 and 0.37 µM against W2), isoprosopilosine (**86**) (IC_{50} 0.13 µM against D6 and 0.24 µM against W2), and juliprosopine (**87**) (IC_{50} 0.36 µM against D6 and 0.60 µM against W2) (Samoylenko et al., 2009). Furthermore, these compounds displayed high selectivity index (SI) to KB cells.

From the methanolic extracts of twigs of *Ficus septica* (Moraceae), active phenanthroindolizine alkaloids dehydrotylophorine (**88**), dehydroantofine (**89**), and tylophoridincine D (**90)** were isolated with antimalarial activity in the IC_{50} range of between 0.03-0.4 µM against *P. falciparum* 3D7. While dehydrotylophorine (**88**) exhibited cytotoxicity effects on mouse fibroblast cells at IC_{50} 8.2 µM, the other compounds were relatively safe at 50 nM (Kubo et al., 2016). In another study, quassi-symmetric bisindole alkaloids strychnogucines A (**91**) and B (**92**) were isolated from *Strychnos icaja* (Loganiaceae) roots. On evaluation of these compounds against CQ-resistant W2 *Plasmodium* strain, Strychnogucine B (**92**) had high potency (IC_{50} 80 nM) though highly cytotoxic (Frédérich et al., 2001). Indole alkaloid dihydrousambarensine (**93**) from *S. usambarensis* had antimalarial activity at IC_{50} 23 nM against W2 and

selectivity index of 1474. Amazingly, when tested *in vivo*, this compound was not active against CQ-sensitive *P. berghei* parasites (Frederich et al., 2008). However, isostrychnopentamine (**94**) from the same plant displayed antimalarial activity against CQ-sensitive and CQ-resistant *P. falciparum* (IC_{50} 120 nM, SI = 60), and *in vivo* against both *P. berghei* and *P. vinkei pefferi* in mice (ED_{50} 30 mg/kg) (Frédérich et al., 2004). In another study, an indolomonoterpenoid alkaloid chrysopentamine (**95**) displayed antimalarial activity against three *P. falciparum* cell lines; F_CA 20, FCB-R and W2 at IC_{50} of ~500 nM.

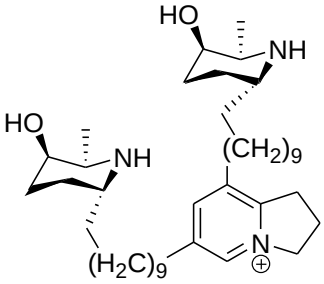
An indoloquinoline alkaloid cryptolepine (**96**) isolated from the leaves of a West African climbing shrub *Cryptolepis sanguinolenta* (Apocynacaeae) harvested from Guinea Bissau had potent *in vitro* antimalarial activity at IC_{50} 0.23 µM against multidrug-resistant K1 strain and IC_{50} 0.059 µM against CQ-sensitive T996 strain compared to CQ's IC_{50} 0.26 µM against K1 and 0.019 µM against T996 (Paulo et al., 2000). An indolobenzazepine alkaloid cryptoheptine (**97**) was second most active from the same plant with IC_{50} values of 0.8 µM against K1 and 1.2 µM against T996. When administered orally, cryptolepine (**96**) suppressed 80% *P. berghei* parasitemia in mice at 50 mg/kg/day (Wright, 2007). The mode of action of compound **96** is postulated to resemble that of CQ by inhibiting hemozoin formation and not DNA intercalation (Onyeibor et al., 2005). Elsewhere, De Andrade-Neto and co-workers isolated potent antimalarial monoterpene indole alkaloids from Brazilian plants. Aspidocarpine (**98**) isolated from *Aspidosperma desmanthum* (Apocynaceae) barks inhibited multidrug-resistant *P. falciparum* K1strain at IC_{50} 0.019 µM while ellipticine (**99**) from *A. vargasii* had IC_{50} values of 0.073 – 0.81 µM against K1 and 3D7 (Andrade-Neto et al., 2007); Rocha e Silva et al., 2012).

Active alkaloids have been isolated from *Flindersia* plants. Flinderole B (**100**) and C (**101**) with antimalarial activity at IC_{50} 0.15 µM and 0.34 µM, respectively, against Dd2 were isolated from *F. amboinensi* (Rutaceae). Isoborreverine (**102**) (IC_{50} 0.32 µM against Dd2) and dimethylisoborreverine (**103**) (IC_{50} 0.08 µM against Dd2) were

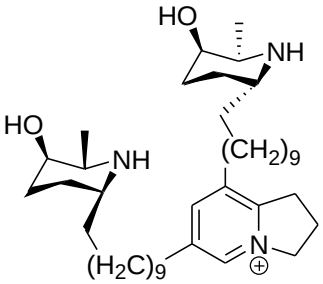
isolated from *F. fournieri* (Fernandez et al., 2009). Voacomine (**104**) is the principal bisindole alkaloid from the Brazilian *Tabernaemontana fuchisiafolia*. This bisindole alkaloid displayed potent antimalarial activity against CQ-resistant *P. falciparum* at IC_{50} 411 nM and SI = 47 and suppressed parasitemia by 43% at 10 mg/kg in Peter's 4-day suppression test (Ramanitrahasimbola et al., 2001). Elsewhere, spermine alkaloids isolated from Kenyan *Albizia gumnifera* had significant antimalarial activity at IC_{50} range of between 0.18-0.24 µM against CQ-sensitive NF54 *P. falciparum* parasites but less potent against CQ-resistant ENT30 (IC_{50} 1.43 - 1.79 µM). When tested *in vivo* against *P. berghei* in mice, Budmanchiamine K (**105**) displayed the highest potency by suppressing 72.9% parasitemia at a dose of 20 mg/kg/day on oral treatment (Rukunga et al., 2007). Samoylenko et al., (2009) isolated a macrocyclic spermine alkaloid 5,14-dimethylbudmunchiamine L1 (**106**) from *Albizia schimperiana* (Leguminosae) that exhibited promising antimalarial activity at IC_{50} 0.27 µM against CQ-sensitive D6 and 0.34 µM against resistant strain W2.

Among the indole alkaloids isolated from the methanolic leaf extract of *Alstonia macrophylla* (Apocynaceae), alstiphyllanine B (**107**) exhibited a promising antimalarial activity at IC_{50} 0.60 µM against CQ-sensitive *P. falciparum* 3D7 (Hirasawa et al., 2009). Naphthylisoquinoline alkaloids from *Triphophyllum peltatum* (Dioncophyllaceae) displayed potent antimalarial activities against NF54 and K1 strains; dioncopettine A (**108**) (IC_{50} 0.021 µM against NF54), dioncophylline A (**109**) (IC_{50} 0.086 µM against K1), dioncophylline B (**110**) (IC_{50} 0.063 µM against K1) and dioncophylline C (**111**) (IC_{50} 0.014 µM against NF54) (François et al., 1996). Upon *in vivo* evaluations, dioncopettine A (**108**) suppressed parasitemia levels close to 100% while dioncophylline C (**111**) completely cured infected mice following oral treatment with 50 mg/kg body weight for 4 days without noticeable toxicity effects (Francois et al., 1997). Elsewhere, Mambu et al., (2000) reported strong antimalarial activity of bisbenzylisoquinoline alkaloids (-)-curine (**112**) (IC_{50} 353 nM) and isochondodedrine (**113**) (IC_{50} 892 nM) isolated from stem barks of *Isolona ghesquiereina*.

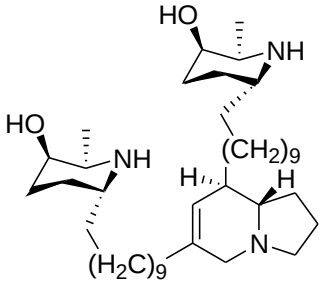
An investigation on antimalarial activities of leaf extracts of *Michelia figo* (Magnioliceae) isolated two active bisbenzylisoquinolines, magnoline (**114**) (IC_{50} 1.5 µM against FCR3 and < 0.16 µM against K1) and magnolamine (**115**) (IC_{50} < 0.16 µM against FCR3 and 1.28 µM against K1) (Phrutivorapongkul et al., 2006). An alkaloid cassarin A (**116**) isolated from the leaves of *Cassia siamea* (Leguminosae) depicted a promising antimalarial activity at IC_{50} 23.5 nM (Morita et al., 2007). Among the four alkaloids isolated from *Stephania rotunda* (Menispermaceae) and tested for antimalarial activities, potent bioactivity was exerted by dehydroroemerine (**117**) (IC_{50} 0.36 µM) and cepharanthine (**118**) (IC_{50} 0.61 µM) (Chea et al., 2010).



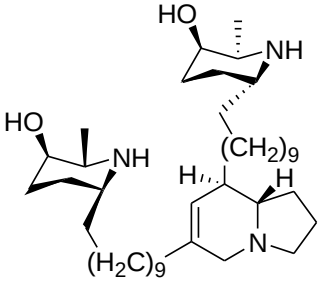
Prosopilosidine (**83**)



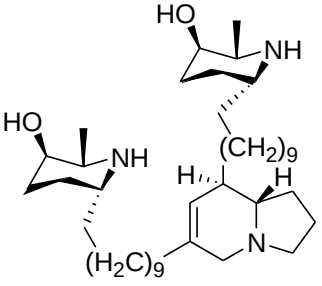
Isoprosopilosidine (**84**)



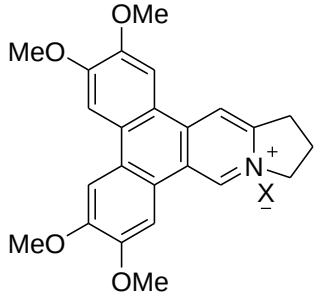
Prosopilosine (**85**)



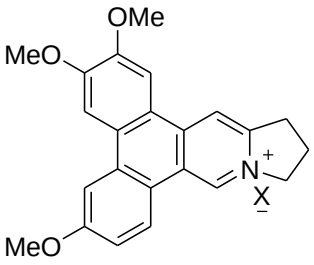
Isoprosopilosine (**86**)



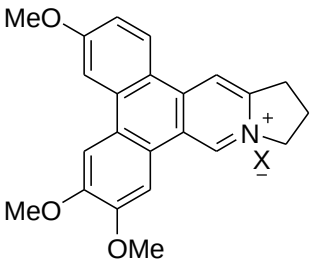
Juliprosopine (**87**)



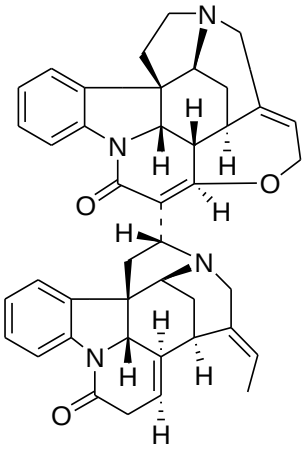
Dehydrotylophorine (**88**)



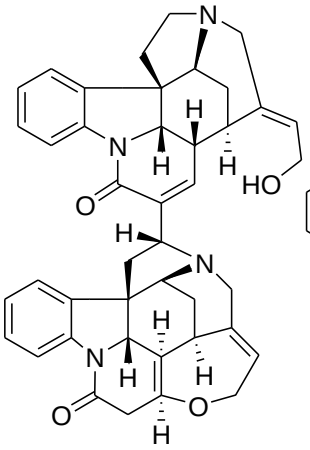
Dehydroantofine (**89**)



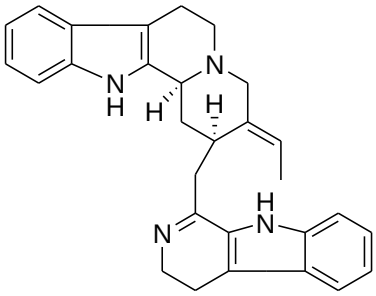
Tylophoridincine D (**90**)



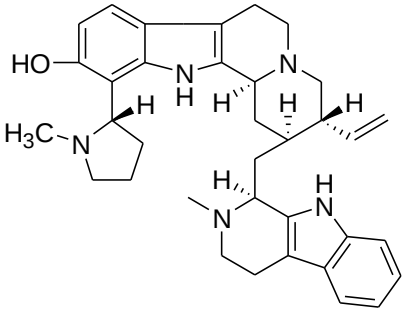
Strychnogucine A (**91**)



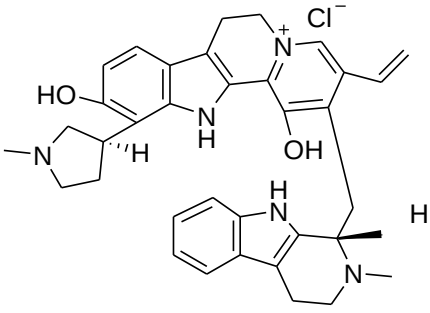
Strychnogucine B (**92**)



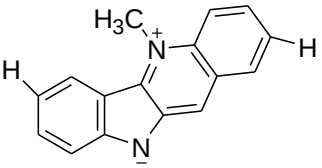
Dihydrousambarensine (**93**)



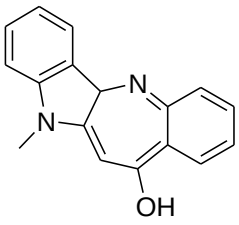
Isostrychnopentamine (**94**)



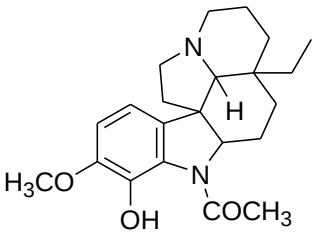
Chrysopentamine (**95**)



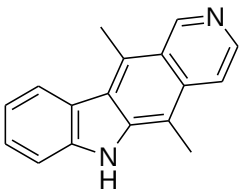
Cryptolepine (**96**)



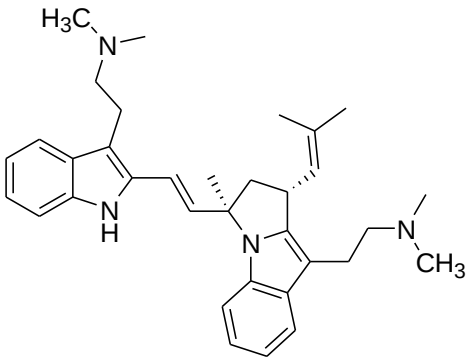
Cryptoheptine (**97**)



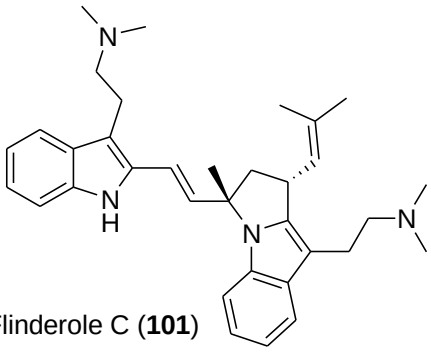
Aspidocarpine (**98**)



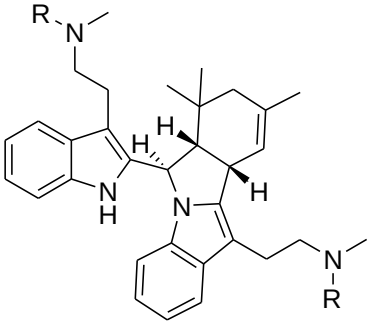
Ellipticine (**99**)



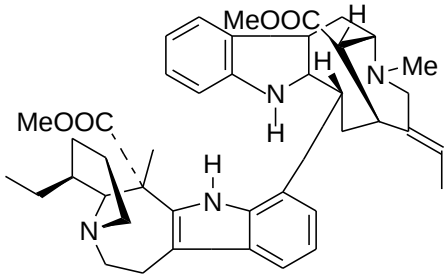
Flinderole B (**100**)



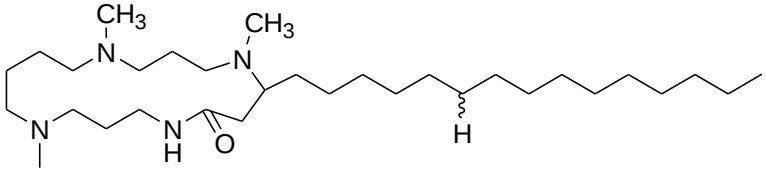
Flinderole C (**101**)



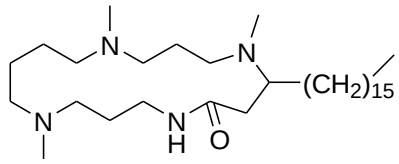
Isoborreverine (**102**) R = H
Dimethylisoborreverine (**103**) R = CH3



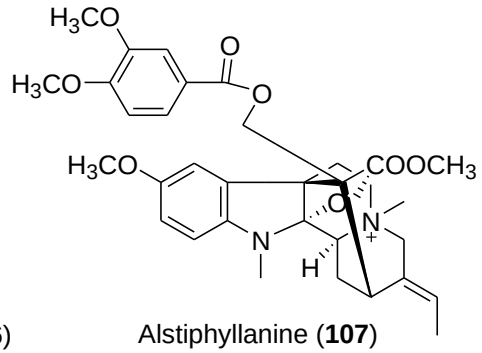
Voacomine (**104**)



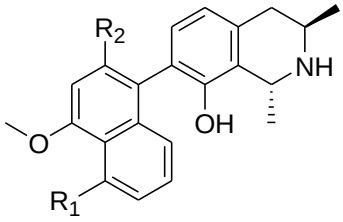
Budmanchiamine K (**105**)



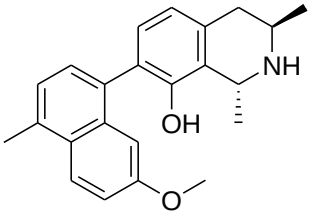
5,14-dimethylbudmunchiamine L1 (**106**)



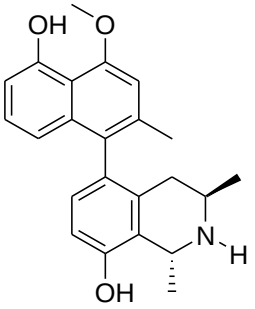
Alstiphyllanine (**107**)



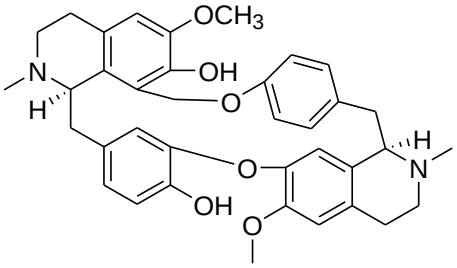
Dioncopettine A (**108**) R1 = OH; R2 = CH2OH
Dioncophylline A (**109**) R1 = OCH3; R2 = CH3



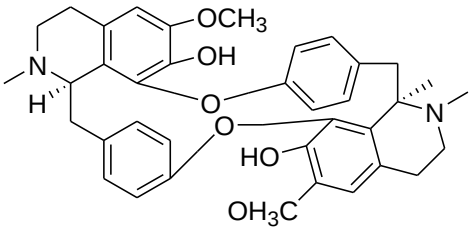
Dioncophylline B (**110**)



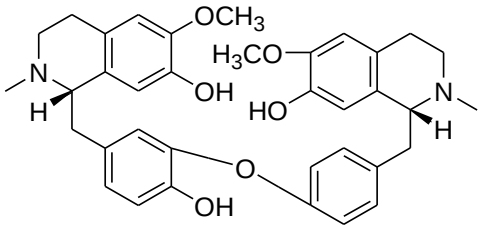
Dioncophylline C (**111**)



(-)-Curine (**112**)



Isochondodedrine (**113**)



Magnoline (**114**)

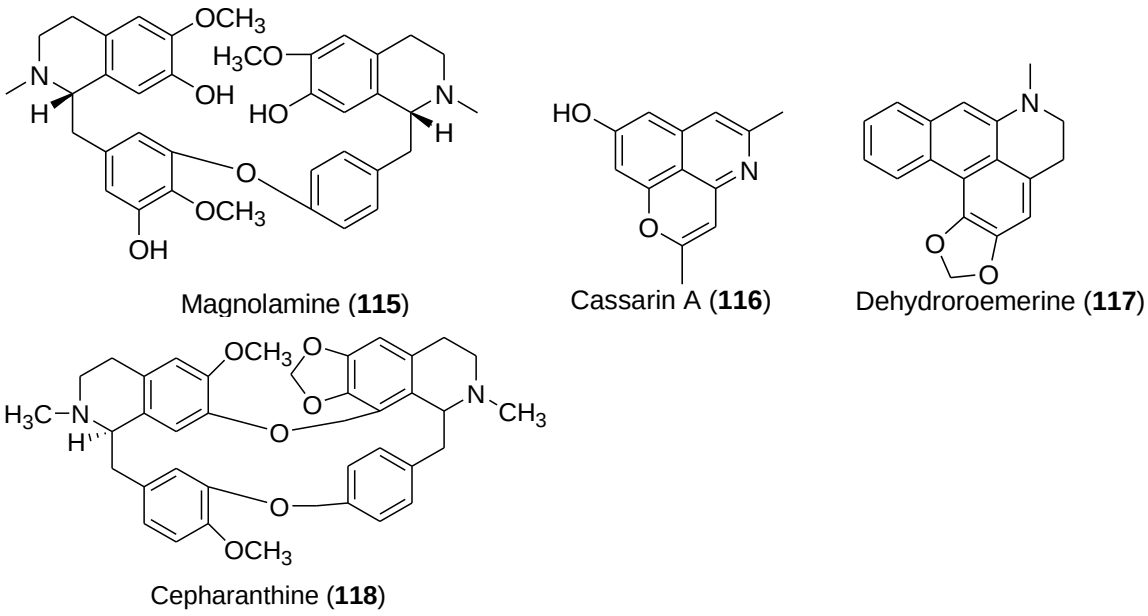


Figure 2.12: Plant-derived antimalarial alkaloids.

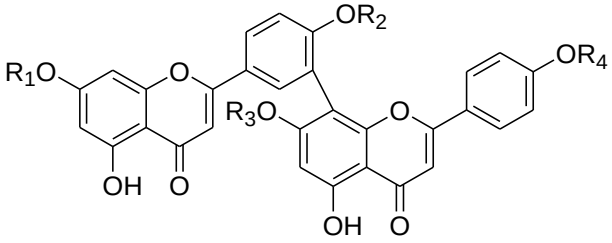
2.4.2 Phenolic derivatives

Several ubiquitous plant phenolic compounds with significant antimalarial activities have been widely reported from various plants (Figure 2.13). Notably, xanthones, flavonoids, cinnamic derivatives and lignans are well-known antimalarial agents. Two (2) bioflavonoids amentoflavones (**119**) and (**120**) isolated from an Indian herb *Selaginella bryopteris* (Selaginellaceae) had *in vitro* antimalarial activity against *P. falciparum* K1 strain at IC_{50} 0.30 and 0.26 µM, respectively (Kunert et al., 2008). De Andrade-Neto and co-investigators isolated four active lignans from *Holostylis reniformis* roots; (7'*R*,8*S*,8'*R*)-3',4,4',5-tetramethoxy-2,7'-cyclolignan-7-one (**121**) (IC_{50} 0.32 µM), (7'*R*,8*R*,8'*S*)-3',4,4',5-tetramethoxy-2,7'-cyclolignan-7-one (**122**) (IC_{50} 0.20 µM), (7'*R*,8*S*,8'*R*)-4,5-dimethoxy-3',4'-methylenodioxy-2,7'-cyclolignan-7-one (**123**) (IC_{50} 0.20 µM) and (7'*R*,8*S*,8'*S*)-3',4,4',5-tetramethoxy-2,7'-cyclolignan-7-one (**124**) (IC_{50} 0.63 µM) against CQ-resistant *P. falciparum* parasites. These lignan compounds exhibited low cytotoxicity on hepatic cells HepG2 A16 and 67% parasitemia reduction *in vivo* (De Andrade-Neto et al., 2007).

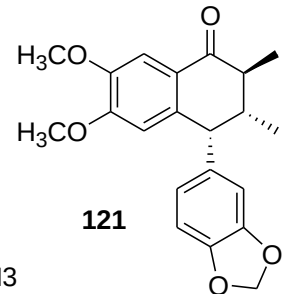
An Indonesian herb *Artocarpus champeden* (Moraceae) has been reported to possess antimalarial prenylated flavones that display differential potent activities against CQ-sensitive *P. falciparum* 3D7 parasites; Artocarpones A (**125**) and B (**126**) (IC_{50} 0.12 and 0.18 µM, respectively), Artonin A (**127**) (IC_{50} 0.55 µM), cycloheterophyllin (**128**) (IC_{50} 0.02 µM), artoindonesianin R (**129**) (IC_{50} 0.66 µM), heterophyllin (**130**) (IC_{50} 1.04 µM), heteroflavanone C (**131**) (IC_{50} 1 nM) and artoindonesianin A-2 (**132**) (IC_{50} 1.31 µM) (Widyawaruyanti et al., 2007). Elsewhere, isoprenylated flavone artopeden A (**133**) from barks of the same plant exhibited antimalarial activity against *P. falciparum* 3D7 at IC_{50} 0.11 µM (Wahyuni et al., 2009).

Green tea (*Camellia sinensis*, Theaceae) leaf extracts contain catechins such as catechin (C) (**134**), gallocatechin (**135**), epicatechin (EC) (**136**), epicatechin gallate (ECG) (**137**), epigallocatechin (EGC) (**138**), and epigallocatechin gallate (EGCG) (**139**). A study conducted by Sharma *et al*., (2007) showed that these flavonoids reversibly inhibited *Plasmodium* enoyl acyl carrier protein reductase (*Pf*ENR) with EGCG (**139**) being highly potent at nanomolar range (K_i = 79 ± 2.67 nM). Furthermore, all the catechins potentiated the binding of triclosan to *Pf*ENR in a two-step mechanism increasing its activity to low picomolar concentration (K_i = 1.9 ± 0.46 pM). A prenylated xanthone derivative tovophyllin A (**140**) was isolated from a methanolic root extract of *Allanblackia monticola* (Clusiaceae). The compound exhibited promising antimalarial activity at IC_{50} 0.7 µM against FCM29, but was less potent against F32 (IC_{50} 20.3 µM) with relatively low cytotoxicity (Azebaze et al., 2006).

From the roots of a Brazilian plant *Pothomorphe peltata* (Piperaceae) and flowers, leaves and roots of *Piper peltatum* (Piperaceae), 4-nerolidylcatechol (**141**) with potent activity at IC_{50} 0.67 µM against multidrug resistant K1 *Plasmodium* strain was isolated (De Andrade-Neto et al., 2007).



119 R1=H; R2=CH3; R3=R4=H
120 R1 = CH3; R2 = H; R3=R4=CH3



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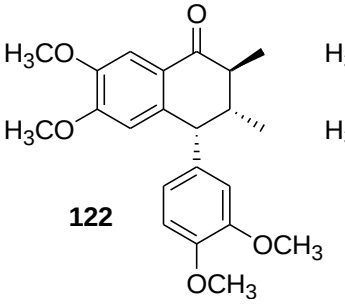

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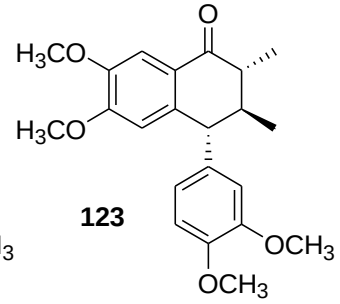

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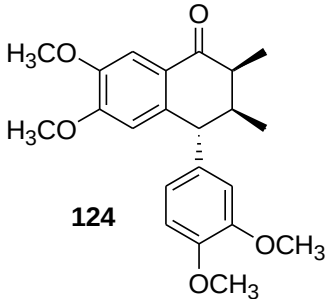

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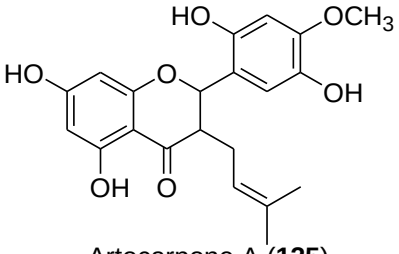
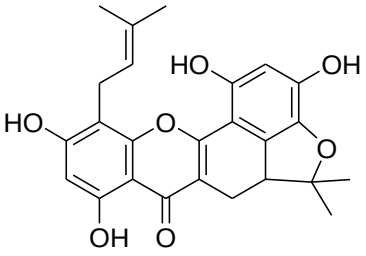
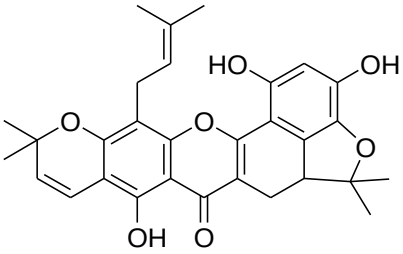


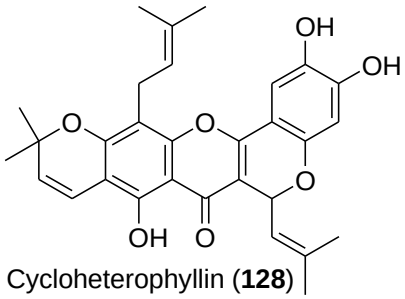
Artocarpone A (**125**)



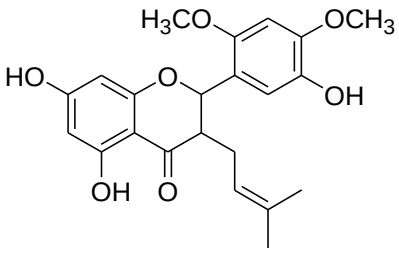
Artecarpone B (**126**)



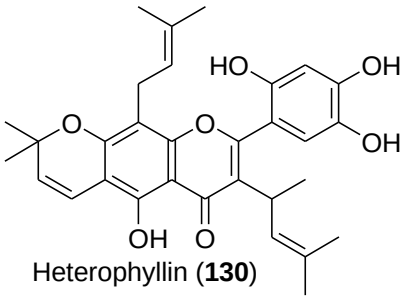
Artonin A (**127**)



Cycloheterophyllin (**128**)



Artoindonesianin R (**129**)



Heterophyllin (**130**)

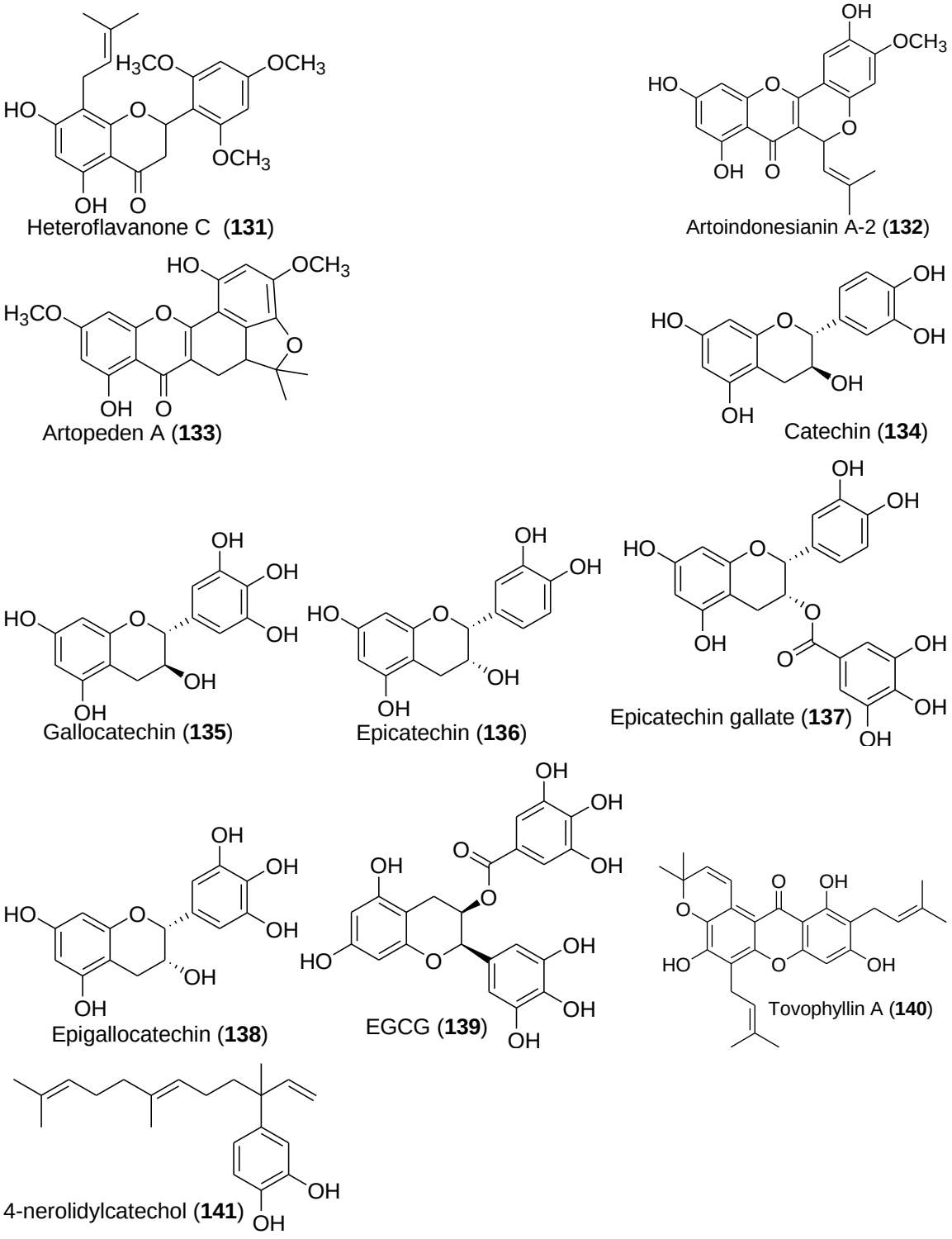


Figure 2.13: Antimalarial phenolic compounds isolated from plants

2.4.3 Terpenoids and quissanoids

Another class of chemical compounds with antimalarial properties is terpenoids that comprise of limonoids, sesquiterpenes, diterpenes, triterpenoids, and quissanoids. Among the triterpenoid limonoids isolated from ethanolic root extract of *Chisocheton ceramicus*, ceramicine B (**142**; Figure 2.14) showed potent activity against *P. falciparum* 3D7 (IC_{50} 0.56 µM) attributed to the presence of a tetrahydrofuran ring at C4/C6 and C28 (Mohamad et al., 2009). In another study, a sesquiterpene lactone ineupatorolide A (**143**) from chloroform soluble fraction of *Carpesium rosulatum* (Asteraceae) displayed potent antimalarial activity against CQ-resistant *P. falciparum* D10 strain at IC_{50} 0.019 µM (19 nM) (Moon, 2007). With this encouraging *in vitro* potency, Korean investigators demonstrated that the compound **143** exhibited potent *in vivo* activity against *P. berghei* in mice at 2, 5, and 10 mg/kg/day displaying a significant blood schizonticidal activity in 4-day early infection and high mean survival rates relative to CQ (5 mg/kg/day) (Chung et al., 2008).

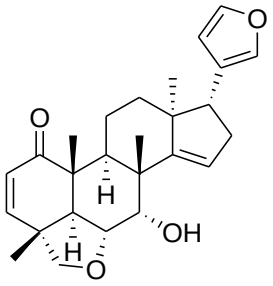
A quissanoid delaumonone A (**144**) from barks of *Laumoniera bruceadelpha* (Simaroubaceae) had strong antimalarial activities at 0.6 µM against *P. falciparum* 3D7 (Oshimi et al., 2009). In another study, bruceolide-type quissanoids from Chinese herbal drug (*Ya dan zi*) prepared from *Brucea javanica* fruits exhibited antimalarial activity, but with high cytotoxicity. In an attempt to improve their selectivity, bruceloide derivatives 3,15-dimethylcarbonate (**145**) and 3,15-diethylcarbonate (**146**) analogues were synthesized and exerted potent antimalarial activities (IC_{50} 90 and 64 nM, respectively) with low cytotoxicity on mouse mammary tumor FM3A cells (SI >700 and 563, respectively). Furthermore, these compounds suppressed *P. berghei* parasitemia at ED_{50} 1.3 and 0.49 mg/kg/day, respectively at doses of 3 mg/kg/day with no obvious toxic effects (Murakami et al., 2004).

During a chemical exploration study of *Camchaya calcarea* (Asteraceae), eight sesquiterpene lactones with moderate antiplasmodial activities against multidrug-resistant K1 strain (IC_{50} 3.3 – 8 µM) were isolated. The most potent compound from this

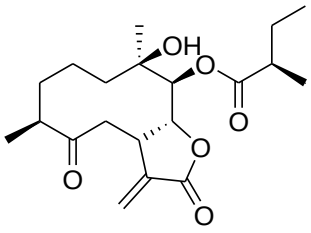
plant was lychnophorolide A (**147**) (IC_{50} 0.8 µM) (Vongvanich et al., 2006). An abietane-type diterpenoid endoperoxide, 13α-*epi*-dioxiabiet-8(14)-en-18-ol (**148**) isolated from petroleum ether leaf extract of Nigerian *Hyptis suavelons* (Lamiaceae) displayed high antimalarial activity at IC_{50} 0.1 µg/mL against D10 (Chukwujekwu *et al*., 2005). Among the sesquiterpene lactones isolated from *Neurolaena lobata* germacranolide sesquiterpenes like neurolenin B (**149**) (IC_{50} 0.62 µM) were more potent compared to furanoheliangolides lobatin B (IC_{50} 16.51 µM) (François et al., 1996). Activity-directed isolation of petroleum ether extract of whole parts of *Viola verecunda* (Violaceae) led to a triterpenoid *epi*-oleanolic acid (**150**) with antimalarial activity against FcB1 (IC_{50} 39 nM) (Moon, Jung, & Lee, 2007). A diterpene norcaesalpinin E (**151**) isolated from *Caesalpinia crista* (Caesalpiniaceae) harvested from Myanmar and Indonesia inhibited *Plasmodium* growth at IC_{50} 90 nM against FCR3/A2 strain compared to CQ (IC_{50} 0.29 µM) (Kalauni et al., 2006).

Quissanoids simalikalactone D (**152**) and E (**153**) and a triterpene picrasin B (**154**) isolated from leaf extract of *Quassia amara* (Simaroubaceae) harvested from French Guiana displayed antimalarial activities against FcB1, F32, W2 strains at IC_{50} range of between 0.01-0.8 µM (Bertani et al., 2006; Cachet et al., 2009). A similar quissanoid simalikalactone D (**152**) alongside another orinocinolide (**155**) both possessing a promising antimalarial activity (IC_{50} 0.0063 - 0.018µM against D6 and W2) were isolated from the root bark of Peruvian *Simaba orinocensis* (Simaroubaceae) (Muhammad et al., 2004). Elsewhere, active antimalarial compounds bisnortriterpene quinone methides 20-*epi*-isoiguesterinol (**156**) (IC_{50} 0.16 µM against D6 and W2) and isoiguesterin (**157**) (IC_{50} 0.5 µM against D6 and 0.42 µM against W2) were isolated from petroleum ether root extracts of *Salacia magadascariensis* (Celasteraceae) (Thiem *et al*., 2005). Other highly potent antimalarial quinone methides from a South African plant *Salacia kraussii* (Celastraceae) were isolated from the root extracts by activity-based fractionation; 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**158**) and isoiguesterol (**159**) (IC_{50} 0.090 µM against K1 and 0.079 µM against NF54). These compounds displayed 30-50 fold *in vitro* antimalarial activity relative to their cytotoxicity in HT-29

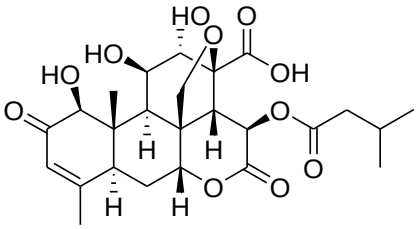
cells (Figueiredo et al., 1998). In another study quissanoid diterpenoids eurycomanone (**160**) and pasakbumin B (**161**) isolated from roots of *Eurycoma longifolia* (Simaroubaceae) displayed strong antimalarial activity against *P. falciparum* W2 and D6 strains at IC_{50} 0.04/0.06 µM against W2/D6 and IC_{50} 0.05/0.08 µM against W2/D6, respectively, but with strong cytotoxicity on human breast cancer (MCF-7) and lung cancer (A549) cells at low concentrations (Kuo *et al.*, 2004). Additionally, quissainoids neosergeolide (**162**) and isobrucein B (**163**) were isolated from roots and stems of another Simaroubaceae plant *Picrolemma sprucei* in Brazil. These compounds were reported to possess strong antimalarial activity at IC_{50} values ranging between 0.002–0.008 µM against multidrug resistant *P. falciparum* K1 strain (Andrade-Neto et al., 2007).



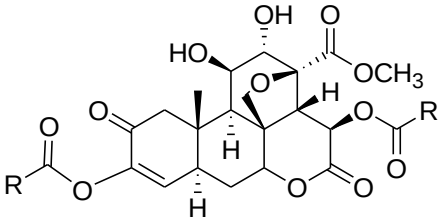
Ceramicine B (**142**)



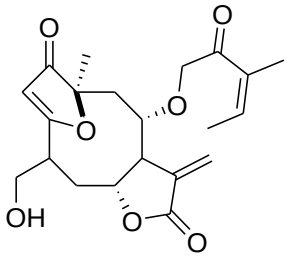
Ineupatorolide A (**143**)



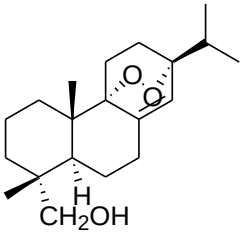
Delaumonone A (**144**)



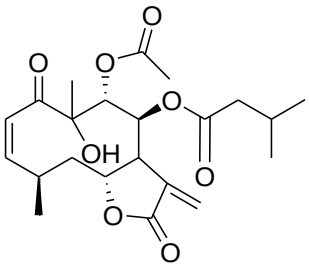
Bruceloide derivative **145** R=OCH3
Bruceloide derivative **146** R =OCH2CH3



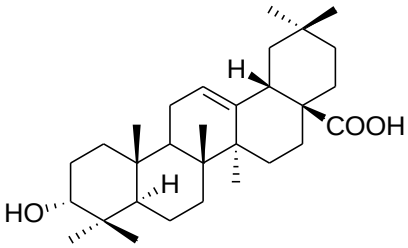
Lychnophorolide A (**147**)



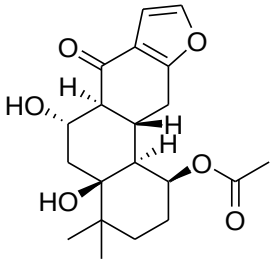
13a-epi-dioxiabiet-8(14)-en-18-ol (**148**)



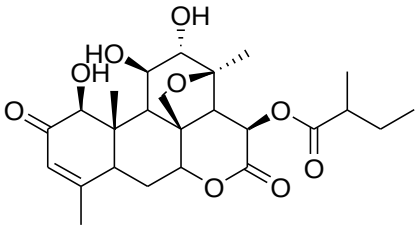
Neurolenin B (**149**)



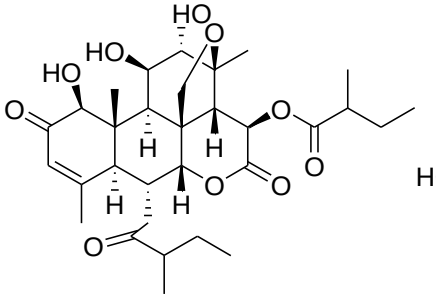
epi-oleanolic acid (**150**)



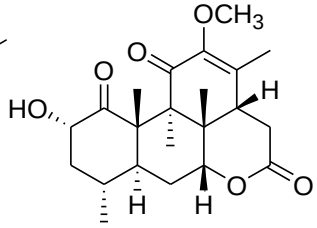
Norcaesalpinin E (**151**)



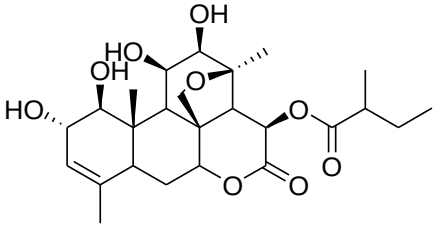
Simalikalactone D (**152**)



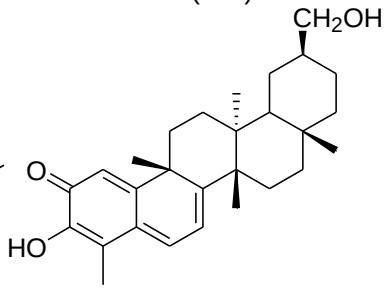
Simalikalactone E (**153**)



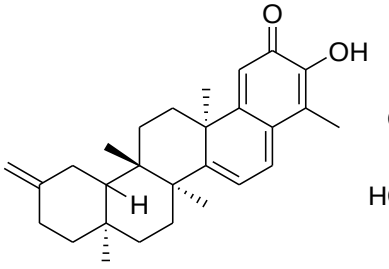
Picrasin B (**154**)



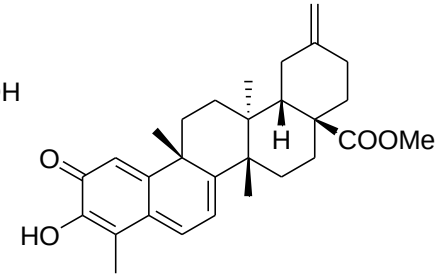
Orinocinolide (**155**)



20-epi-isoiguesterinol (**156**)



Isoiguesterin (**157**)



17-(methoxycarbonyl)-28-nor-isoiguesterin (**158)**

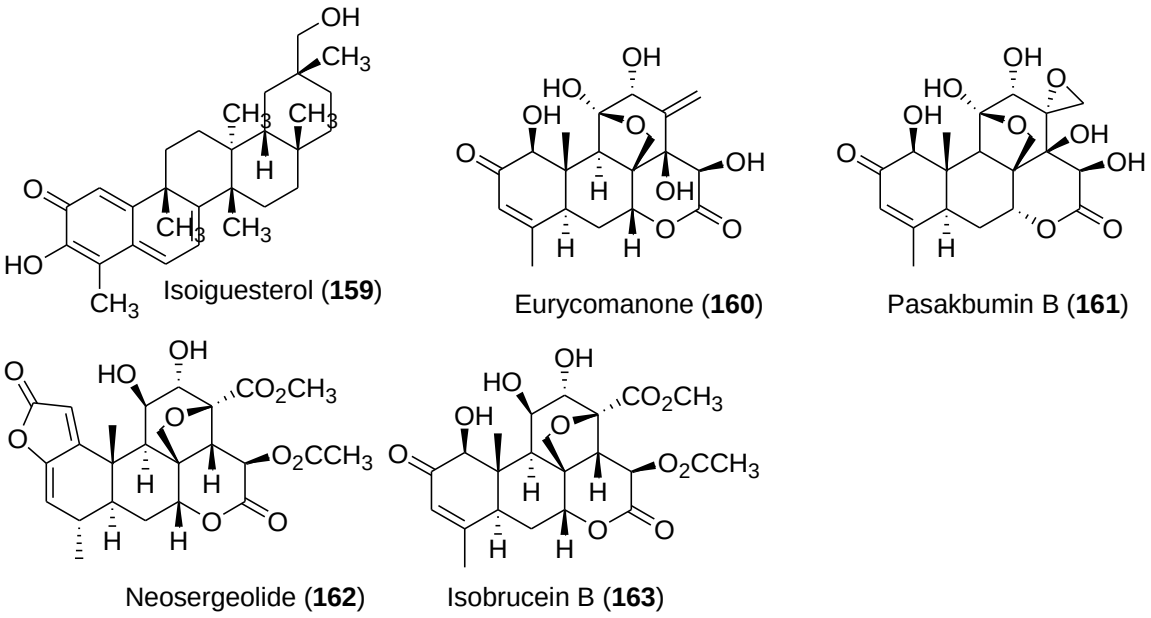
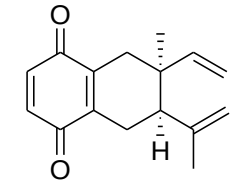


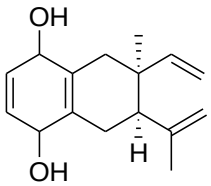
Figure 2.14: Potent antimalarial terpenoid compounds derived from plants.

2.4.4 Quinones

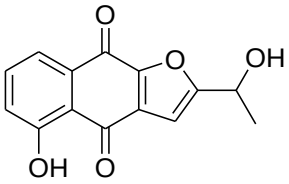
Dettrakul et al. (2009) isolated active quinones cordiachrome C (**164**; Figure 2.15) and cordiaquinol (**165**) from *Cordia globifera* (Boraginaceae) with antimalarial activity at IC_{50} 0.8 µM and 1.2 µM against multidrug resistant *Plasmodium* K1 parasites. Morais and collaborators isolated dihydroxyfuranonapthoquinones 5 (**166**) & 8-hydroxy hydroxyethy naptho[2,3-b]furan-4,9-diones (**167**) from the bark of Brazilian plant *Tabebuia incana* (Bignoniaceae). These compounds exhibited potent antimalarial activities against FcB2 at IC_{50} 0.67 µM (Morais et al., 2007). From the roots of the African *Bulbine frutescens* (Asphodelaceae), two dimeric phenylanthraquinones joziknipholones A (**168**) and B (**169**) with promising antimalarial activities at IC_{50} 164 nM and 270 nM, respectively against K1 were isolated (Bringmann et al., 2008). A napthoquinone plumbagin (**170**) with antimalarial activity at IC_{50} 0.27 µM was isolated from a Thailand plant *Nephenthes thorelii* (Nephenthaceae) (Likhitwitayawuid et al., 1998).



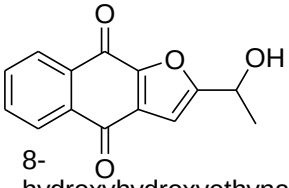
Cordiachrome C (**164**)



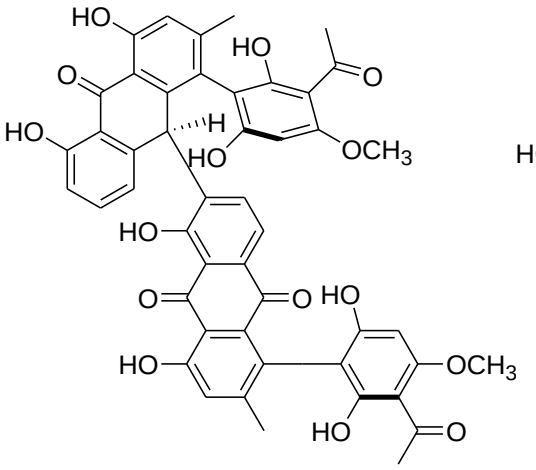
Cordiaquinol (**165**)



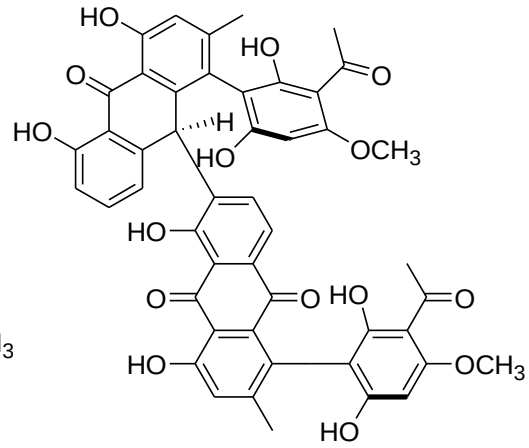
Dihydroxyfuranonapthoquinone 5 (**166**)



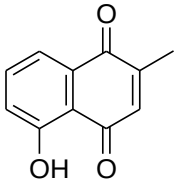
8-hydroxyhydroxyethynapth o[2,3-b]furan-4,9-dione (**167**)



Joziknipholone A (**168**)



Joziknipholone B (**169**)



Plumbagin (**170**)

Figure 2.15: Antimalarial quinone derivatives isolated from plants.

Other miscellaneous compounds with promising antimalarial activities are polyacetylenes isolated from the root bark of a Tanzanian plant *Cussonia zimmermanii* (Araliaceae); 8-hydroxyheptadeca-1-ene-4,6-diyn-3-yl acetate (**171**; Figure 2.16) (IC_{50} 0.44 µM) and 11,16-diacetoxyoctadeca-17-ene-12,14-diynyl acetate (**172**) (IC_{50} 0.84

µM) (Senn et al., 2007). Additionally, potent amino steroid sarachine (**173**) isolated from leaves of *Saracha punctata* displayed strong *in vitro* antimalarial activity (IC_{50} 25 nM) and suppressed 83% *P. vinckei* parasitemia in mice at 100 mg/kg/2 days (Moretti et al., 1998).

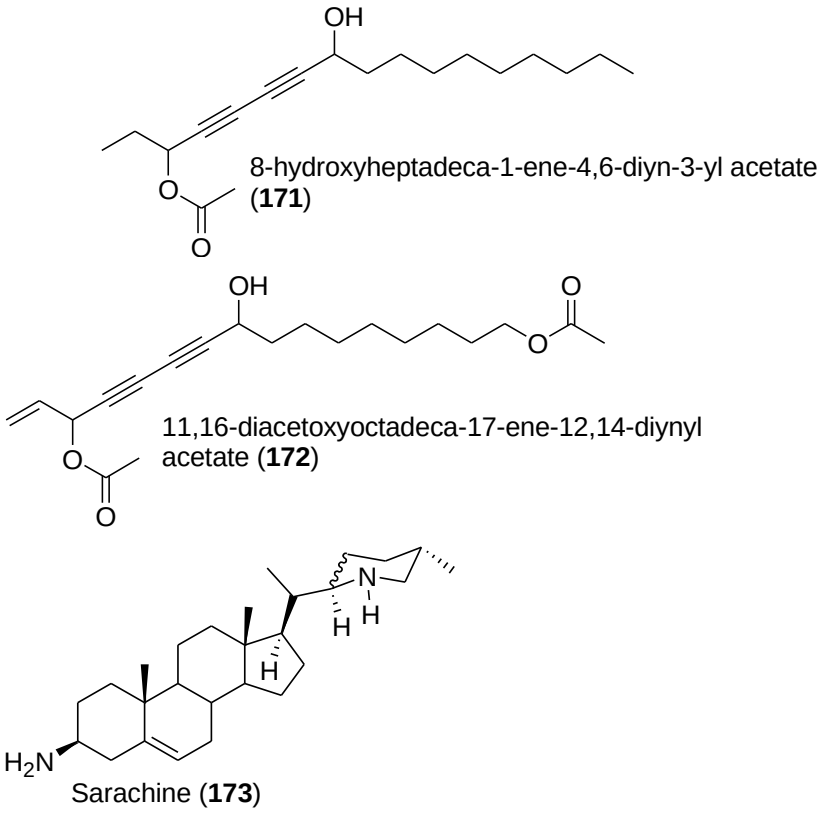


Figure 2.16: Miscellaneous potent antimalarial plant-derived compounds.

While these antimalarial compounds have demonstrated promising contribution to drug development for treatment of malaria, majority have limited or no capacity to kill the transmissible stages of *Plasmodium*.

2.5 Transmission-blocking interventions in pursuit of malaria elimination

2.5.1 Transmission-blocking vaccines (TBVs)

In order to drive the malaria elimination goal into its realization, devoid of effective tools, the discovery of new arsenals is an unrefuttable mission to venture (Alonso et al.,

2011). A number of studies have identified the agonistic role played by a few potential vector- and parasite-specific surface proteins and their blockade could sufficiently reduce transmission (Delves, Angrisano, & Blagborough, 2018). Unlike other malaria vaccines that directly benefit the patient by either limiting the infection and/or clinical symptoms, transmission-blocking vaccine intervention instead prevents onward mosquito transmissions by blocking sporozoites development and malaria prevalence in communities (Carter et al., 2000; Draper et al., 2018; Sandeu et al., 2016). The naturally acquired antibodies from the immunizations are perceived to bind onto the male and female gametocytes, eventually reducing mosquito infectivity rates in malarous populations. From "-omics" standpoint, several candidate proteins (~325) are expressed on gametocytes surface as well as on ookinetes, but only 10 characterized antigens have been proven immunogenically viable and 5 are lead TBV candidates.

The well-characterized known parasite-specific immunogenic proteins include *Pfs*25/28 (homologous to *Pbs*25/28), WARP, Chitinase, CTRP, Hap2, and PSOP12 expressed on ookinetes (Li et al., 2004; Sala et al., 2015; Angrisano et al., 2017), and *Pfs*48/45, *Pfs*230, and *Pfs*47 expressed on the membrane surface of gametocytes (Duffy & Kaslow, 1997; Molina-Cruz et al., 2013). While many antigenic peptides may lose antigenicity *in vivo*, stability appears to be linked to the nature of conjugation although reduction in efficacy over successive rounds of transmission due to parasite antigenic variation upon pressure to similar immune challenge is undoubtedly possible (Blagborough et al., 2013). Fascinating findings from preclinical and Phase I vaccine trials of a viable *Pfs*25 show that the vaccine could sustain high immunogenicity when conjugated to EPA/Alhydrogel adjuvants and reduce mosquito infections (Kapulu et al., 2015; Talaat et al., 2016; Bompard et al., 2017). Unexpectingly, bivalent TBVs containing *Pfs*25 and either *Pfs*28 or *Pfs*230 did not offer substantial advantage to reductions in transmissions when compared to monovalent strategies (Menon et al., 2018).

Some candidate TBVs target the glycosylated polysaccharides within the mosquito midgut that functionally aid ookinete invasion by masking the glycan ligands and

subsequently arresting ookinete-oocyst maturation. Antibodies directed against midgut carboxypeptidase B (CPBAg1) were demonstrated to block post-fertilization *P. falciparum* and *P. berghei* oocyst maturation when imbibed into mosquito blood meals or fed directly on an infected mice immunized with recombinant CPBAg1 (Lavazec et al., 2007). Elsewhere, identification of alanyl aminopeptide N in *An. gambiae* midgut (AnAPN1) and subsequent targeting with monoclonal and polyclonal antibodies abrogated ookinete development of *P. berghei* and *P. falciparum* in different mosquito species (Dinglasan, Kalume, et al., 2007). In fact, this candidate target (AnAPN1) was chosen for further vaccine development and evaluations by the PATH Malaria Vaccine Initiative (MVI) in collaboration with John Hopkins Bloomberg School of Public Health and Sabin Vaccine Institute (www.path.org; Atkinson et al., 2015). Recently, a midgut fibrinogen-related protein, FREP1 (also known as FBN9), was established to display a broad spectrum antigenicity against *Plasmodium* parasites and anti-FREP1 antibody blocked >75% and >81% *P. berghei* and *P. falciparum* transmissions to *An. gambiae*, respectively, without inducing noticeable immunopathology (Niu et al., 2017). In another study, monoclonal antibodies AC-43 and AC-29 targeting uncharacterized midgut glycoproteins were found to remarkably reduce *P. vivax* oocysts densities by 78.6% in Indian *An. culicifacies* mosquitoes, suggesting potential TBV targets (Chugh et al., 2010).

As a viable TBV could suppress malaria transmissions by magnitude folds, the feasibility to such a discovery just like pre-erythrocytic and erythrocytic malaria vaccines translates to a long period of waiting. Furthermore, since it depends with the ability of immunized individuals to mount an effective immune response which is negatively impacted by poor health and malnutrition status in malaria endemic regions as well as possible parasite antigenic variation, transmission-blocking drugs offer an alternative to prevent downstream development in mosquitoes.

2.5.2 Antimalarial agents with transmission-blocking potential

Massive investment towards the discovery of antimalarial agents with potent gametocytocidal and transmission-blocking activities particularly from international community in collaboration with public-private partnerships created platforms to accelerate disease control (Birkholtz et al., 2016; Burrows et al., 2017). Some chemical compounds are regarded as purely gametocytocidal, while others exhibiting dual activity i.e. possessing both gametocytocidal and transmission-blocking activities. The clinically approved drug with such dual activities is an 8-aminoquinoline primaquine (PQ, **51**) believed to affect the parasite's mitochondria. But, owing to its profound life-threatening side effects in G6PD patients, expansive use of compound **51** as antimalarial against relapsing *P. vivax* infections is restricted (Baird & Hoffman, 2004; Ganesan et al., 2012). Randomized clinical trials however show that administration of a single small dose (0.25 mg/kg) of **51** or methylene blue (**81**) when added to ACT treatments was sufficient to reduce gametocytemia levels in asymptomatic children with insignificant changes in methanoglobin levels (Gonçalves et al., 2016; Dicko et al., 2018). Efforts to develop much safer and potent compounds from **51** as chemotype have afforded Tafenoquine (TQ, **52**) and primaquine-thiazolidinones (Aguiar et al., 2017).

Albeit of the parent scaffold Artemisinin (**61**) and its ACT partners being unable to clear gametocytes whilst promoting their transmission (Beshir et al., 2013), its derivatives dihydroartemisinin (DHA, **62**), Artemether (**64**), and Artesunate (**65**) have gametocytocidal activities *in vitro* (Kumnuan et al., 2013). These compounds limitedly inhibit early stages I-III exerting little activity if not totally inactive against stages IV-V gametocytes (Adjalley et al., 2011). Use of these compounds as antimalarials is further exacerbated by increasingly growing resistance. Though still not inevitable, but a recent study however reported improved pan-reactive **61** derivatives, Artemisone (**79**) and Artemiside (**174**), that potently inhibited both asexual and late-stage V gametocytes at low nanomolar doses (IC_{50} ~2 nM) by imparting redox imbalance on combination with methylene blue (**81**) (Coertzen et al., 2018). On incubation with a hydroxynaphthoquinone Atovaquone (ATQ, **60**) at 1.4×10^{-7} M for 96 h, substantial

reductions in stage-specific NF54 gametocyte development were achieved at 75% for stage II, 54% for stage III, and 20% for stage IV, but 14% increase in stage V population (Fleck et al., 1996).

Focusing into the next-generation of antimalarials, through high throughput screening (HTS) of MMV, GSK-Tres Cantos Antimalarial Set (GSK-TCAMS), Dundee protein kinase scaffold, St. Jude Children's research hospital, and Norvatis malaria box libraries, a number of transmission-blocking compounds have been discovered (Figure 2.17). On screening 13,533 compounds from GSK-TCAMS against *P. falciparum* stage V gametocytes, Almela and co-workers identified 98 compounds with dual activity against asexual and gametocyte stages. But, the only 6 selected molecules – TCMDC-123475 (**175**), TCMDC-125849 (**176**), TCMDC-125114 (**177**), TCMDC-125487 (**178**), TCMDC-125133 (**179**), and TCMDC-137453 (**180**) with gametocytocidal effects at 0.12 to 1.17 µM were found to inhibit >88% male exflagellation and > 95% blockade to mosquito transmission (Almela et al., 2015). From the same chemical library, Miguel-Blanco et al., (2017) identified 400 compounds possessing late-stage gametocytocidal activity at IC_{50} < 2 µM and 69% (276/400 compounds) displaying dual asexual/gametocytes activity. From a selection of 6 compounds only 4 –TCMDC-123767 (**181**), TCMDC-141698 (**182**), TCMDC-141070 (**183**), and TCMDC-141154 (**184**) exerted >80% exflagellation and transmission-blocking when tested at 1 - 2 µM. From 3,825 Norvatis-GNF Malaria Box compounds, four hexahydroquinolines GNF-Pf-5640 (**185**), GNF-Pf-5660 (**186**), GNF-Pf-5668 (**187**), and GNF-Pf-5310 (**188**) were identified to possess potent *in vitro* early/late stage gametocytocidal activities with **188** losing activity during mosquito feeding assays (Vanaerschot et al., 2017). Sanders et al., (2014) screened 1,500 and 400 compounds from FDA approved library and MMV malaria box library, respectively, identifying 44 compounds with gametocytocidal activity at submicromolar IC_{50} values. Eight (8) compounds; pyrvinium pamoate (**189**), pentamidine (**190**), maprotiline (**191**), anastrozole (**192**), pyrithione zinc (**193**), 1-pentanol (**194**), anozolene sodium (**195**), and benzalkonium chloride (**196**) exhibited gametocytocidal activities at IC_{50} < 1 µM and only **189** exhibited 100% transmission-

blocking efficacy at 500 nM. Recently, 13,983 compounds from various libraries were screened for gametocytocidal activities and > 90% of known antimalarial compounds were inactive against late-stage gametocytes, supporting Adjalley et al., (2011) findings. Only amino alcohols derivatives; lumefantrine (**50**), halofantrine (**49**), mefloquine (**47**), and aromatic methylene blue (**81**) had late-stage V activity (IC_{50} < 0.5 µM). Furthermore, the screen validated gametocytocidal activities of previously reported compounds; NITD609 (**70**), GNF179 (**197**), KAI407 (**198**), KDU691 (**199**), KAF246 (**200**), DDD107498 (**73**), and pentamidine (**190**). Three MMV candidate compounds MMV667491 (**201**), MMV019881 (**202**), and MMV665882 (**203**) displayed EC_{50} < 0.5 µM (Plouffe et al., 2016).

In their study to identify compounds with transmission-blocking activities, Delves et al., (2018) screened 68,689 compounds from Global Health Chemical Diversity Library from which 17 potent molecules inhibited transmissions. Four (4) compounds were male gamete-specific inhibitors, while seven (7) were macrogamete-specific and 6 exhibited dual activity. From a selection of 5 active compounds; DDD599/BPCA (**204**), DDD291 (**205**), DDD504 (**206**), DDD968 (**207**), and DDD881 (**208**), only **205** and **208** completely blocked oocyst development. Using similar approach, Sun et al., (2014) screened 5,215 repurposed drug molecules against *P. falciparum* 3D7 gametocytes and 27 compounds displayed potent activity of IC_{50} < 1 µM (Table 2.3). This depicts that some compounds with other biological activities could affect gametocyte functionality as observed with antituberculosis isonicotinic acid hydrazide (**209**) (Arai et al., 2004) and (A/T)AAA DNA binding anticancer agent centanamycin (**210**) (Yanow et al., 2008). In another study, out of 45,056 compounds sourced from Sytravon library 23 exerted dual gametocyte inhibitory (IC_{50} < 10 µM) and transmission-blocking activities against stages III-V NF54 parasites (Sun et al., 2017). Sadly, considering the proportion of output from these screens a bottleneck figure appears for a low number entering antimalarial development pipeline. Currently, only NITD609 (Cipargamin, **70**), KAF156 (**71**), and DDD107498 (**73**) with gametocytocidal effects identified through similar approach are under clinical trials.

Studies show that inhibition of kinase signaling impairs parasite survival and subsequent transmissions, with blockade of *Pf*CDPK4 and *Pf*CDPK5 inhibiting microgamete exflagellations (Ojo et al., 2012; Huang et al., 2016). In one study, imidazopyrazines KDU691 (**199**) exhibited multistage and multistrain antimalarial activity by inhibiting lipid kinase (phosphatidylinositol-4-OH kinase, PI(4)K) activity (McNamara et al., 2013). In another study, targeted inhibition of cyclic GMP-dependent protein kinase (*Pf*PKG) identified ML10 (**211**) (IC_{50} 160 pM) from imidazopyridine series that had cleared asexual blood stages (EC_{50} 2.1 nM) and blocked transmission of late-stage NF54 gametocytes to *Anopheles stephensi* mosquitoes (Baker et al., 2017). Elsewhere, aminopyridine and aminopyrazine derivatives targeting kinase inhibition exerted IC_{50} < 100 nM against asexual blood stages and early-stage NF54 gametocytes (van der Watt et al., 2018).

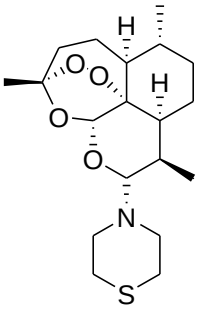
Antibiotics such as Azithromycin (**69**) and ribosomal-targeting thiostrepton derivatives inhibit gametocyte apicoplast development with treated parasites failing to establish in the mosquito midguts (Shimizu et al., 2010; Aminake et al., 2011). When female mosquitoes were offered gametocytemic blood meal imbibed with **69**, *P. falciparum* parasite load in the mosquito midguts was decreased and reduced vector lifespan, underscoring a drug candidate for malaria elimination (Gendrin et al., 2015). From lichens, (+)-Usnic acid derivatives abrogated zygote-ookinete maturation in mosquito midgut blocking transmissions (Pastrana-mena et al., 2016).

Antimalarial iron chelators deprive *Plasmodium* parasites iron required for growth. In quest to investigate the effects of iron chelators on *P. falciparum* sexual stage V infectivity, (S)3''-(HO)-desazadesferrithiocin-polyether [DADFT-PE] (FBS0701) (**212**) was established to prevent mosquito transmissions by inactivating the male and female gametocytes. Furthemore, when administered to infected mice compound **212** killed early stage gametocytes and inhibited asexual stages with a single dose of 100 mg/kg curing *Plasmodium yoelii* infected mice (Ferrer et al., 2012; Ferrer et al., 2015).

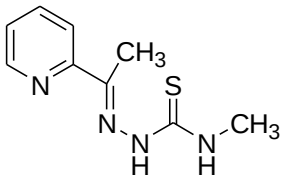
Table 2.3: List of repurposed drug compounds with promising *P. falciparum* gametocytocidal activity of IC_{50} < 1 µM

Compound name	Gametocytocidal activity IC_{50} (µM)	Primary activity
NSC174938 (**213**)	0.003	Anticancer
Torin 2 (**214**)	0.008	Anticancer
Carfilzomib (**215**)	0.012	Anticancer
Dactinomycin (**216**)	0.015	Anticancer, Antibacterial
NVP-AUY922 (**217**)	0.047	Anticancer, Antibacterial
Maduramicin (**218**)	0.047	Antiprotozal
Narasin (**219**)	0.050	Antiprotozal, Antibacterial
Artesunate (**65**)	0.059	Amebicide, Antimalarial
Artemether (**64**)	0.073	Antimalarial
Alvespimycin (**220**)	0.074	Anticancer
Artenimol (DHA) (**62**)	0.077	Antimalarial
Omacetaxine (**221**)	0.083	Anticancer
Thiram (**222**)	0.083	Antifungal
Zinc pyrithione (**193**)	0.093	Antifungal
Phanquinone	0.109	Antibacterial, Antimalarial
Bortezomib	0.118	Anticancer
Artemisinin (**61**)	0.148	Antimalarial
Salinomycin sodium	0.194	Antibacterial, Antiprotozal
Monensin sodium	0.254	Antimalarial, Antiprotozoal
Dipyrithione	0.263	Antibacterial, Antifungal
Dicyclopentamethylenethiuram disulphide	0.274	Other
Methylene blue (**81**)	0.307	Antimalarial, Anticancer
Quinine hemisulfate	0.345	Antimalarial, Analgesic, Anti-inflammatory
YM155	0.372	Anticancer
Withaferin A	0.372	Anticancer
Adriamycin	0.526	Anticancer
Romidepsin	0.637	Anticancer
AZD-1152-HQPA	0.743	Anticancer
CAY 10581	0.743	Anticancer
Mefloquine (**47**)	0.833	Antimalarial, Anti-inflammatory
Plicamycin	0.833	Antibiotics, Anticancer

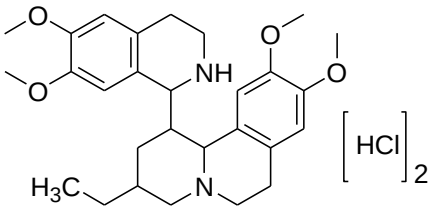
CUDC-101	0.833	Anticancer
Auranofin	0.935	Antirheumatic, Anti-inflammatory
Trametinib	0.935	Anticancer
GSK-458	0.935	Anticancer
Afanitib	0.935	Anticancer
Panobinostat	0.935	Anticancer



Artemiside (**174**)



TCMDC-123475 (**175**)



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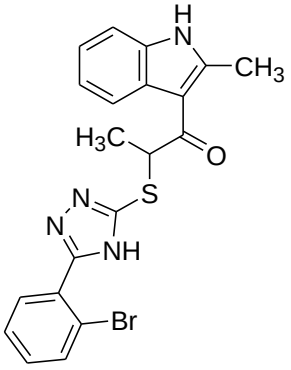

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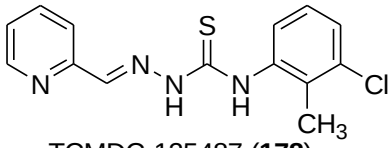

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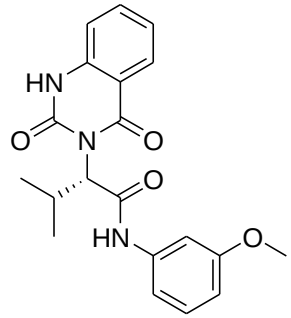
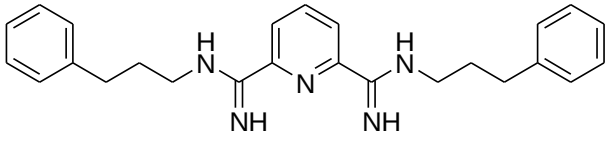
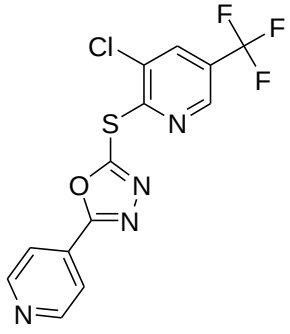


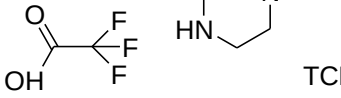
TCMDC-125133 (**179**)

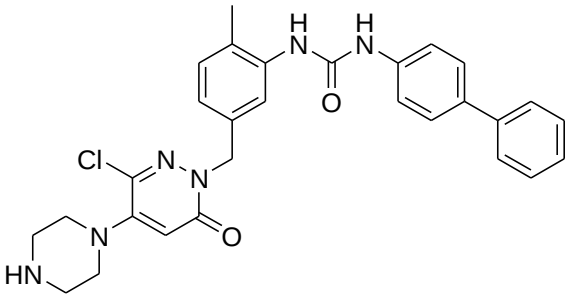


TCMDC-137453 (**180**)

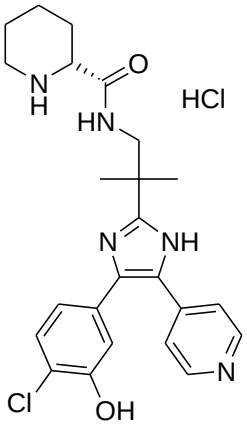


TCMDC-123767 (**181**)





TCMDC-141698 (**182**)



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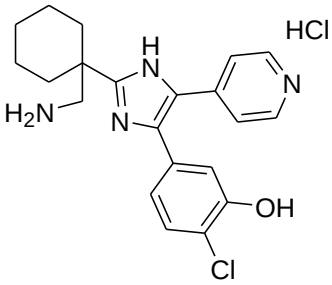

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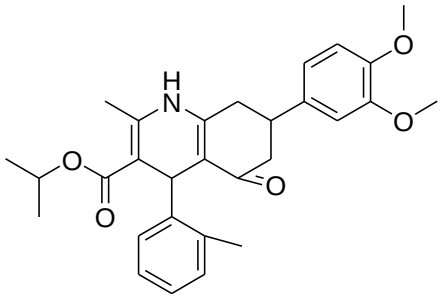
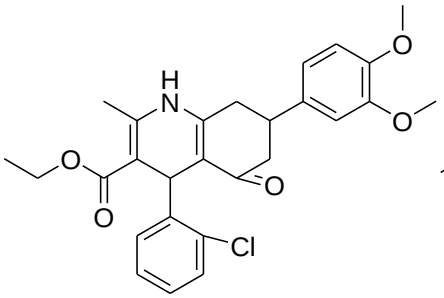


GNF-Pf-5640 (**185**)



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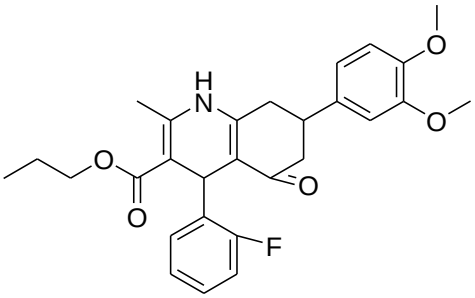
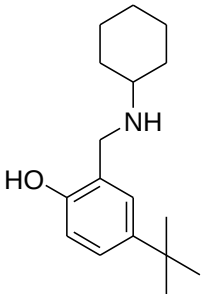
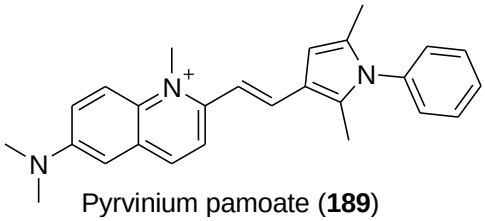


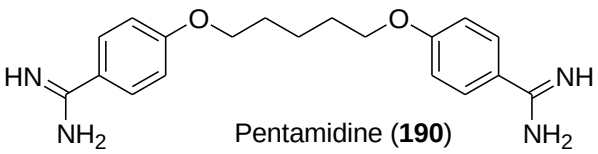
GNF-Pf-5668 (**187**)



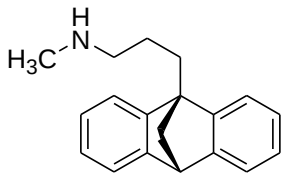
GNF-Pf-5310 (**188**)



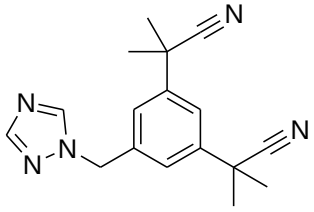
Pyrvinium pamoate (**189**)



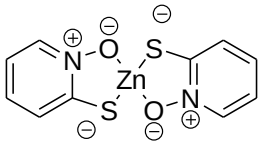
Pentamidine (**190**)



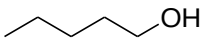
Maprotiline (**191**)



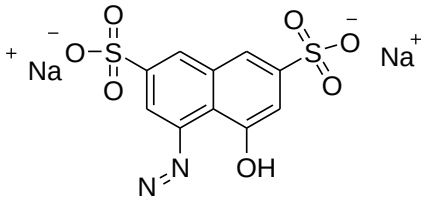
Anastrozole (**192**)



Pyrithione zinc (**193**)



1-pentanol (**194**)



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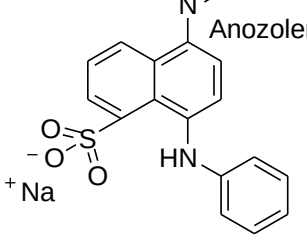
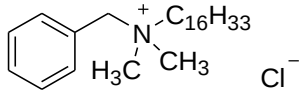
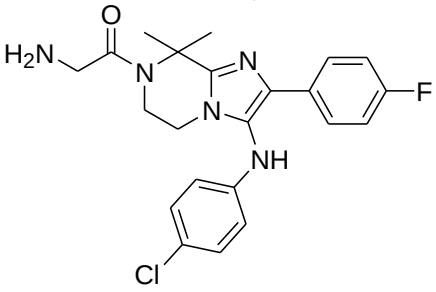




Benzalkonium chloride (**196**)



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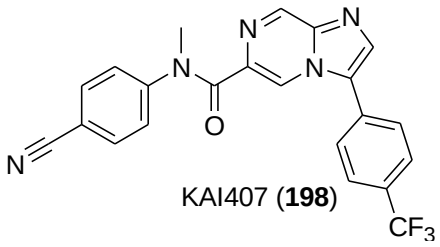

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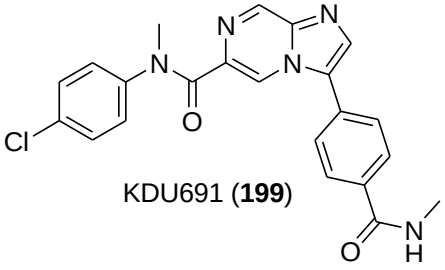

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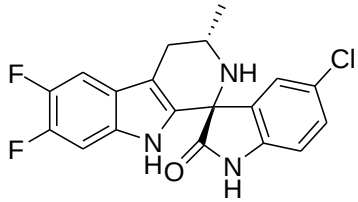

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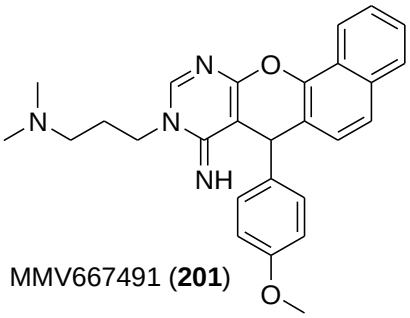
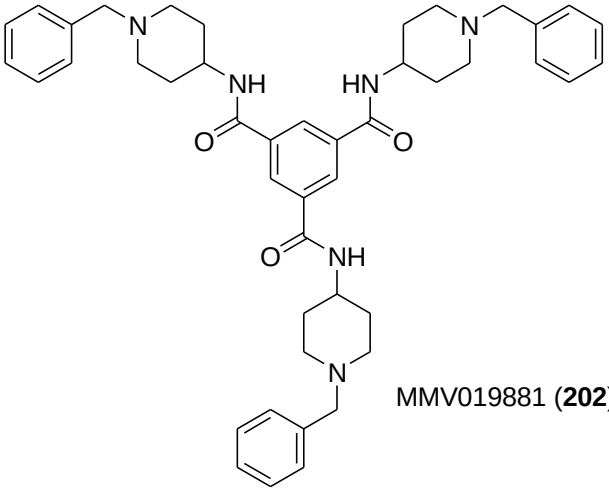
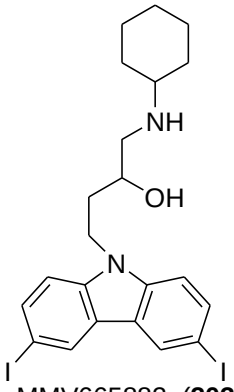


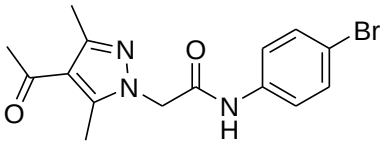
MMV667491 (**201**)



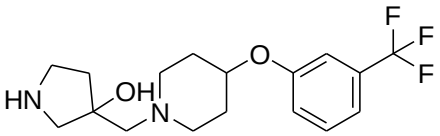
MMV019881 (**202**)



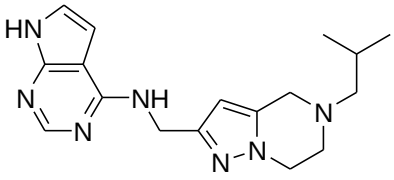
MMV665882 (**203**)



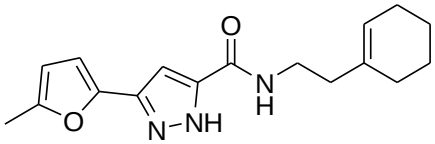
DDD599 (**204**)



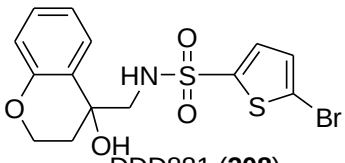
DDD291 (**205**)



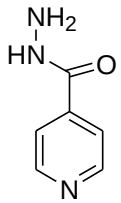
DDD504 (**206**)



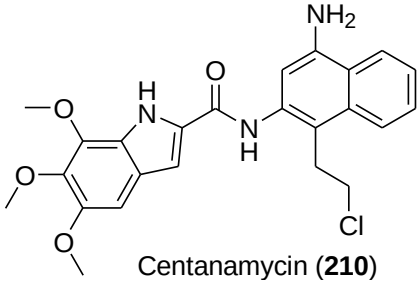
DDD968 (**207**)



DDD881 (**208**)



Isonicotinic acid hydrazide (**209**)



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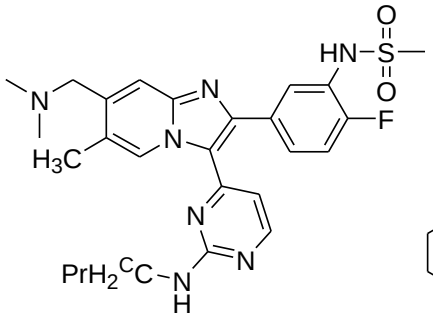
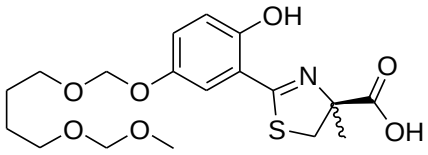
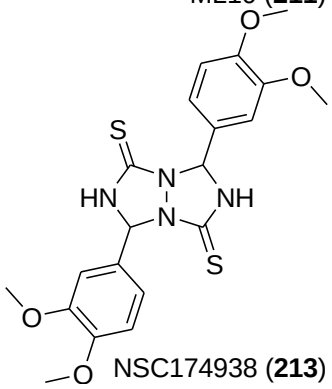


ML10 (**211**)



FBS0701 (**212**)



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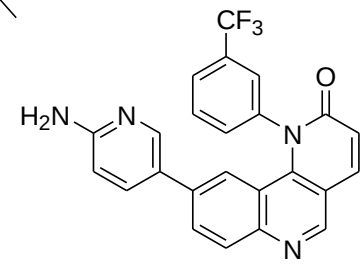
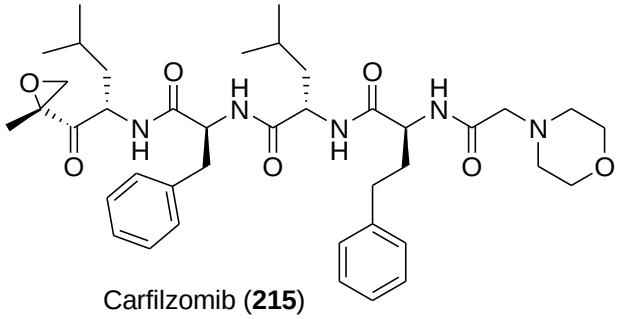
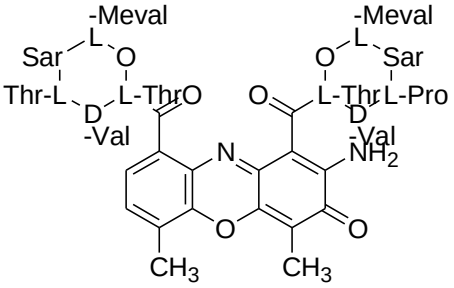


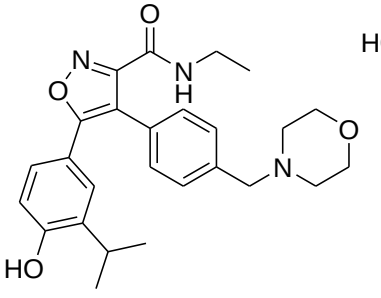
Torin 2 (**214**)



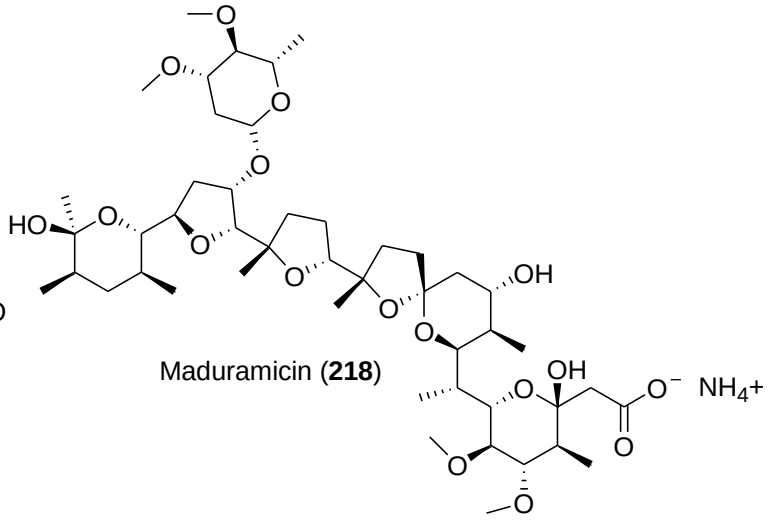
Carfilzomib (**215**)



Dactinomycin (**216**)



NVP-AUY922 (**217**)



Maduramicin (**218**)

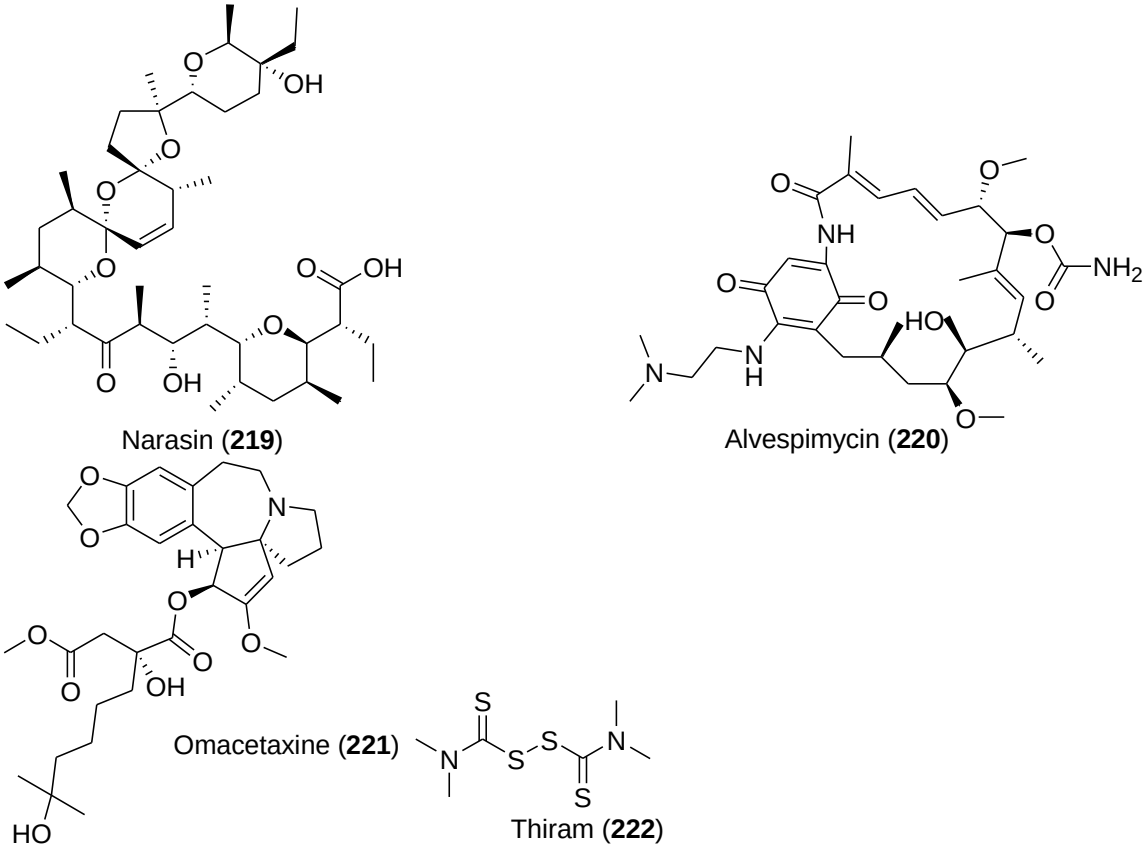


Figure 2.17: Chemical structures of gametocytocidal and transmission-blocking compounds.

2.5.3 Plant-derived transmission-blocking agents

The ensuing motivation from historical discovery of effective plant-derived antimalarials has over the recent years drawn remarkable embarkment in search of transmission-blocking agents based on the community-level prophylactic effects bestowed in certain plant herbal preparations as well as from known insecticidal compounds. For instance, two herbal products containing *Cryptolepis sanguinolenta* (Apocynaceae) and *Carapa procera* (Meliaceae) (CM), and *Aloe schweinfurthii* (Aloaceae), *Khaya senegalensis* (Meliaceae), *Piliostigma thonningii* (Fabaceae), and *Cassia siamea* (Leguminosae) (RT) vendored in Ghanaian markets were identified to possess late-stage gametocytocidal activities (> 80%) when tested at 100 µg/mL (Amoah et al., 2015). Without further

isolation of the bioactive compounds from ethnopharmacogically used South African plant extracts, Moyo and colleagues identified four of 8; *Artemisia afra* (Asteraceae), *Trichilia emetica* (Meliaceae), *Leonotis leonurus* (Lamiaceae) and *Turraea floribunda* (Meliaceae) with > 50-70% inhibitory activities against early and late *P. falciparum* NF54 gametocytes at 10-20 µg/mL (Moyo et al., 2016). These studies suggest presence of yet-to-be explored compounds with potential transmission-blocking activities.

Currently, only a few pure plant-derived compounds have displayed proven potential to inhibit *Plasmodium* gametocytogenesis and sporogonic development (Figure 2.18). As early as 1994, *Azadirachta indica* (Meliaceae) triterpenoid derivatives that also possess insect growth regulatory activities particularly Azadirachtin A (**43**) demonstrated potential developmental inhibition of sexual stages by impairing microgametogenesis through a disrupted mitotic spindle formation (Jones et al., 1994; Billker et al., 2002). Further investigations by Italian researchers demonstrated promising transmission-blocking activities of Azadirachtin A (**43**) (IC_{50} 17 µM), its standardized herbal preparation NeemAzal® (IC_{50} 6.8 µg/mL), and crude extract with detrimental effects on mosquito physiology (Lucantoni et al., 2006; Lucantoni et al., 2010; Yerbanga et al., 2014; Dembo et al., 2015; Dahiya et al., 2016; Tapanelli et al., 2016). From an invasive weed *Parthenium hysterophorus* (Asteraceae) that ecologically support mosquito survival by its sesquiterpene lactone parthenin (**223**) (Nyasembe et al., 2015) and parthenolide (**224**) from *Tanacetum cinerariifolium* (Asteraceae), 100% abrogation of *P. falciparum* ookinete-to-oocyst maturation at 50 µg/mL (191 µM) and 4 µM, respectively, was reported from these two compounds (Balaich et al., 2016). Another elemanolide sesquiterpene lactone vernodalol (**225**) isolated from *Vernonia amygdalina* (Asteraceae) leaves exerted 70-90% *Plasmodium* sporogonic inhibition at IC_{50} 4.2 µg/mL (19 µM) (Abay et al., 2015).

Bioactivity-guided fraction of methanolic extracts from a Tunisian plant *Daucus virgatus* (Apiaceae) aerial parts led to isolation of a potent angeloylated germacranolide daucovirgolide G (**226**) that exhibited 92% sporogonic inhibition of *P. berghei* at 50

µg/mL (IC_{50} 82.3 µM) (Sirignano et al., 2017). Although the activity against sporogonic stages has not been assessed, cryptolepine (**96**) from a West African herbal plant *C. sanguinolenta* (Apocynacaeae) highly inhibited *Pf*NF54 gametocytes (IC_{50} 1965 nM) *in vitro* and showed synergistic effectiveness with Amodiaquine (**48**) (Forkuo et al., 2017).

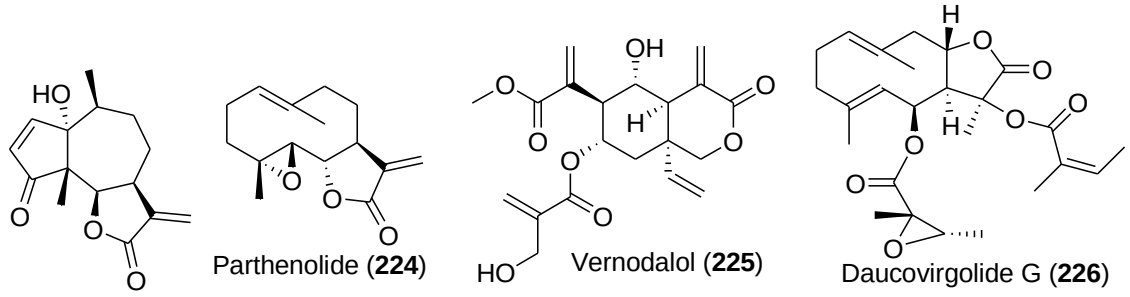
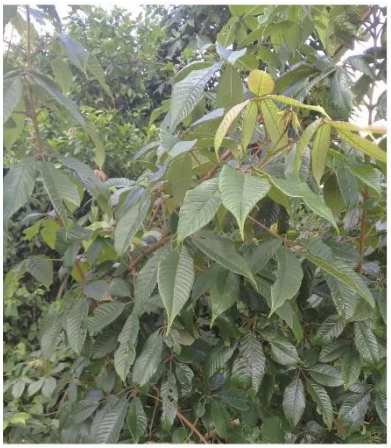


Figure 2.18: Transmission-blocking compounds isolated from plants.

2.6 Phytochemistry and bioactivities of selected plants in the present study

This study focused on the following 13 plants; *Vitex schiliebenii, Vitex payos, Schinus terebinthifolius, Mangifera indica, Zanthoxylum chalybeum, Chrysanthemum cineriariifolium, Cissampelos pariera, Prosopis juliflora, Terminalia brownii, Murraya koenigii, Persea americana, Camellia sinensis,* and *Agerantum conyzoides* (Figure 2.19 A - M). The plants were selected based on chemotaxonomic and previously reported bioactivity criteria: (i) presence of compounds previously reported to inhibit parasite development (Gessler et al., 1994; Hata et al., 2011; Kamaraj et al., 2014) without further investigation on gametocytocidal effects, and (ii) mosquitocidal compounds with a particular signal pathway modulation (Nyamoita et al., 2013; Muema et al., 2017). Although potential bioactivities of the plant extracts have been reported, there is no existence of scientific evidence of their malaria transmission-blocking activities. It was screen for novel bioactive agents with malaria transmission-blocking potential from these plants. A focus was placed on two plants (*C. pareira* and *P. juliflora*) that showed highly potent activity against *P. falciparum* W2 and a clinical isolate MDH 0038 as well as *Z. chalybeum* as a source of larvicides.



A

B

C

D

E

F

G

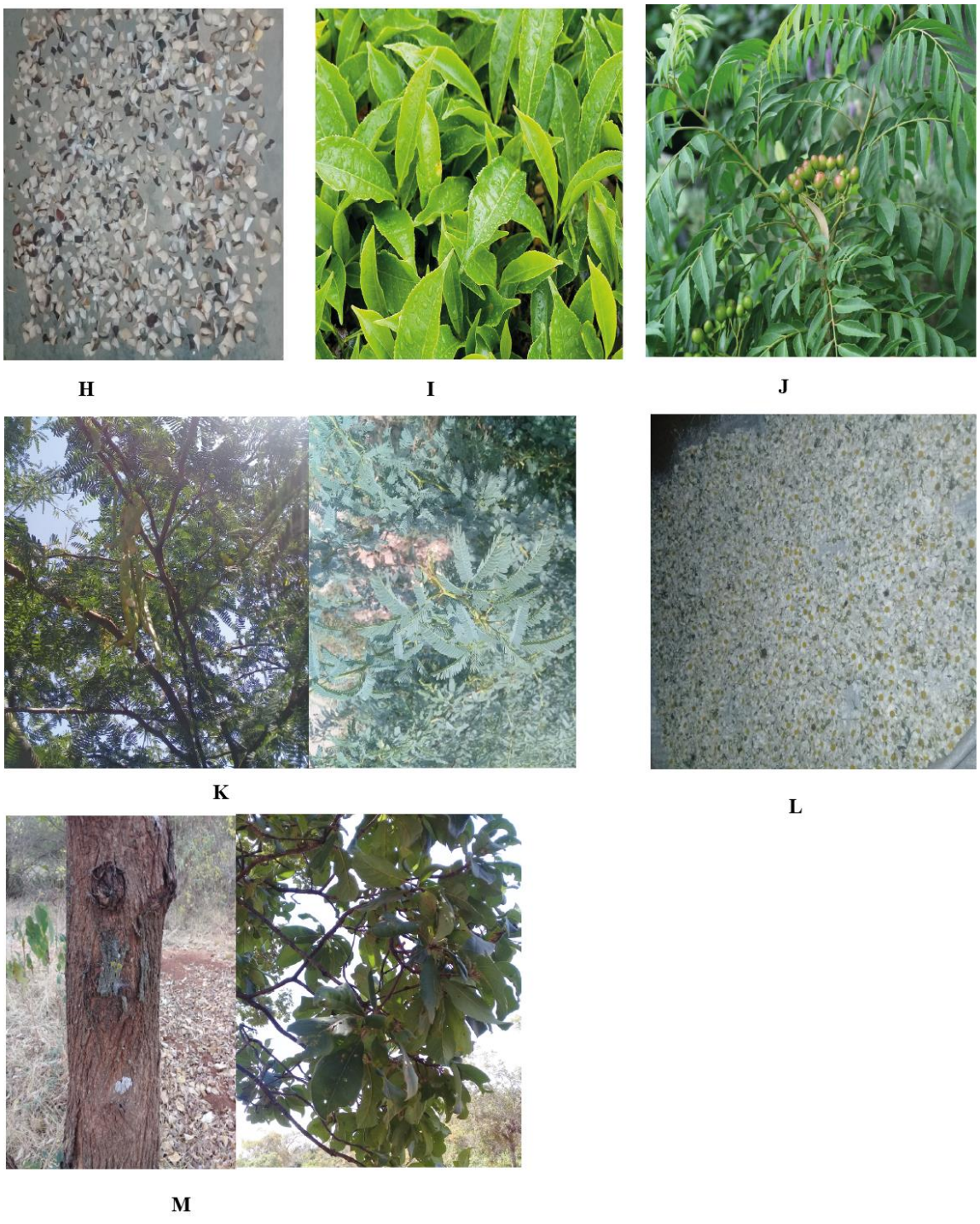


Figure 2.19: Photos of plants under study. (A): *V. schiliebenii*, **(B):** *A branch of V. payos*, **(C):** *Leaves and fruits of S. terebinthifolius*, **(D):** *Stem and leaves of Mangifera indica,* **(E):** *Z. chalybeum*, **(F):** *C. pariera and its roots*, **(G):** *A. conyzoides*, **(H):** *Seeds of P. americana*, **(I):** *Leaves of C. sinensis*, **(J):** *M. koenigii*, **(K):**

P. juliflora with ripe pods, **(L):** *Dry flowers of C. cinerarifolium*, and **(M):** *Stem and leaves of T. brownii* (Source: *J. Muema, 2018*).

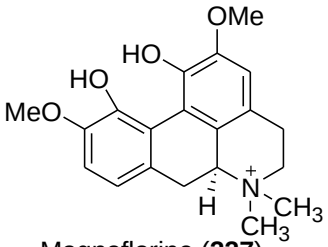
2.6.1 *Cissampelos pariera*

C. pariera Linn is among the 450 species of *Menispermaceae* family that comprises *Stephania gabra*, *S. japonica*, *Tinospora sinensis*, *T. cordiflora*, *T. crispa*, and *Cocculus hirsutus*, among others. These plants have been used widely as folk medicine in India and tropical African countries for their structurally varied isoquinoline alkaloids, anciently used as arrow poisons (Semwal *et al*., 2014). Within the genera *Cissampelos*, 30-40 species distributed across the tropical and subtropical world (including *C. capensis*, *C. mucronata*) have been taxonomically classified. Many are dioecious vine climbers that produce inedible grape-sized fruits. In South American countries; Peru, Brazil, Ecuador, and Columbia, the plants commonly known as *Abuta* are planted to beautify home gardens. Locally, *C. pariera* is commonly known as *"Kakutu*" and "*Karigi-Kanonongwe"* in Kamba and Mbeere dialects, respectively. The alcoholic preparations from the roots have been traditionally documented to treat stomach upsets, alleviate inflammation, relieving abdominal and menstrual pains, snake bite antidote, uterine relaxant (Kokwaro, 1993).

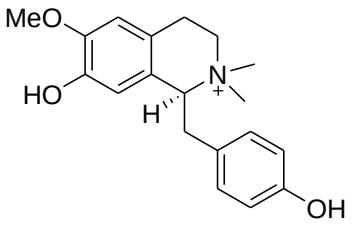
Njeru *et al*., (2015) evaluated the antimycobacterial activity of methanolic extract and solvent fractions of *C. pariera* roots. Although that study did not characterize the major bioactive components, the solvent fractions exhibited strong antituberculous inhibition at MIC range of between 6.25 to 50 µg/mL. Additionally, the extracts showed broad spectrum antimicrobial activities against clinically important pathogenic bacterial and fungal isolates. In the event of screening Indian Ayurveda using cell-based assays, Sood *et al*., (2015) established that alcoholic extracts of *C. pariera* aerial shoots strongly inhibited cell entry of all the four tested serotypes of dengue viruses (DENV) (IC_{50} 1.23 – 78 µg/mL) with selectivity index (SI) of > 45. *In vivo* treatment evaluations in AG129 mice showed significant (p = 0.021) anti-DENV protection at dosage of 250 mg/Kg body weight. These pan-DENV inhibitory effects were found to be exerted by down-regulation of pro-inflammatory TNF-α cytokine secretion at IC_{50} 6.1 µg/mL. A similar antiviral activity has been reported in *C. sympodialis* (Leite et al., 2016).

In their study, Amresh *et al*., (2007) demonstrated that *C. pariera* roots possessed antinociceptive, anti-inflammatory and antiarthritic activities. The bioactivities of *C. pariera* are usually associated with benzylisoquinoline alkaloids. Bala *et al*., (2017) identified various isoquinoline alkaloids from chloroform and *n*-butanol fractions. These included magnoflorine (**227**; Figure 2.20), magnocurarine (**228**), hayatinine (**229**), cycleanine (**230**), cissamine (**231**), (-)-curine (**112**), and a new benzylisoquinoline alkaloid (**232**). Among these compounds, **229** was found highly potent against KB and A549 cancer cells. Other bioactive compounds reported from this plant are (-)-bebeerine (**233**), hayatidine (**234**), cissampareine (**235**), cyclanoline (**236**), (+)-tetrandrine (**237**), dicentrine (**238**), dehydrodicentrine (**239**), grandirubrine (**240**), norimeluteine (**241**), cissampeline (**242**), insularine (**243**), isoimerubrine (**244**), pareirubrine A (**245**) and B (**246**), D-quercitol (**247**), norruffscine (**248**), dimethyltetrandrinium (**249**), pareitropone (**250**) (Kupchan et al., 1960). (-)-bebeerine (**233**) has antimicrobial activity. The compound **237** has been reported to possess antitumor (Wu et al., 2010), antifungal (Zhao et al., 2013), antifibrotic (Hsu et al., 2007), and anti-inflammatory activities (Wu & Ng, 2007).

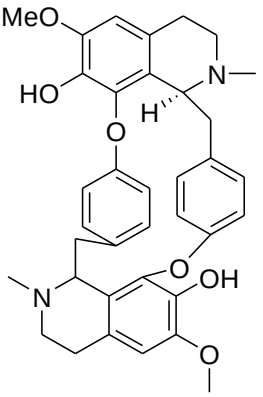
A dimeric chalcone-flavone, cissampeloflavone (**251**), isolated from *C. pariera* aerial parts showed good antitrypanosomatid activity against *Trypanosoma cruzi*, (causative agent of Chagas disease) and *T. brucei rhodesiense* (human African trypanosomiasis) with low cytotoxicity to human KB cells (Ramírez et al., 2003). Furthermore, *in silico* molecular docking of **251** into *T. cruzi* sirtuins; *Tc*SIR2rp1 and *Tc*SIR2rp3 demonstrated a relatively good inhibitory effect (Sacconnay et al., 2014). *In vitro* antimalarial activities of a related species *C. mucronata* against *P. falciparum* have been reported in Tanzania at IC_{50} < 10 µg/mL (Gessler et al., 1995) and were attributed to **112** (IC_{50} 0.24 µg/mL against D6 and 0.22 µg/mL against W2) and a phytosteroid (-)-stigmasterol (**252**) (IC_{50} >5.8 µg/mL against D6 and W2) (Omole, 2011). In contrast, a study conducted in Democratic Republic of Congo associated antimalarial activities of this plant with bisbenzylisoquinone alkaloids, cissamptin (IC_{50} 2596 ng/mL) and isoliensinine (**253**) (IC_{50} 257 ng/mL) (Tshibangu *et al*., 2003).



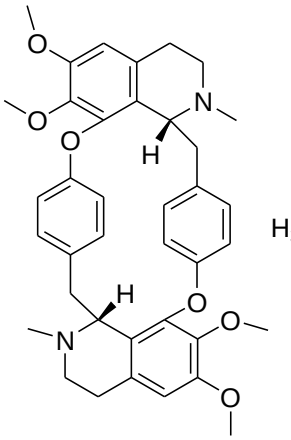
Magnoflorine (**227**)



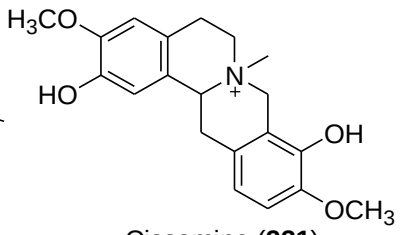
Magnocurarine (**228**)



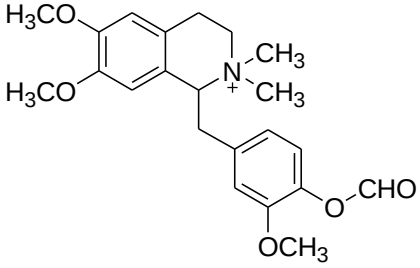
Hayatinine (**229**)



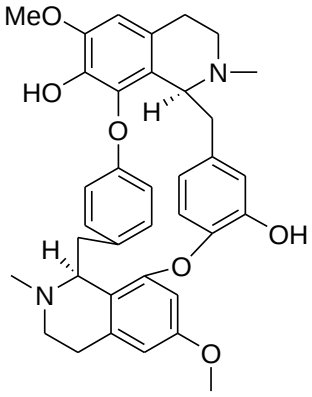
Cycleanine (**230**)



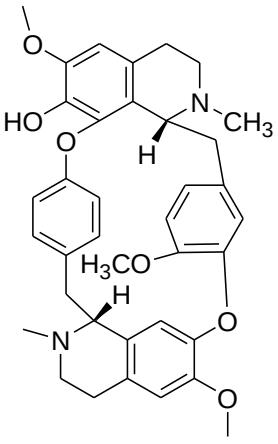
Cissamine (**231**)



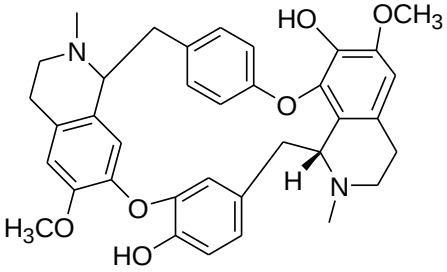
New BBIQ alkaloid (**232**)



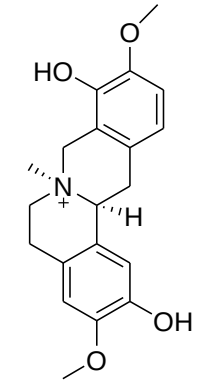
(-)-bebeerine (**233**)



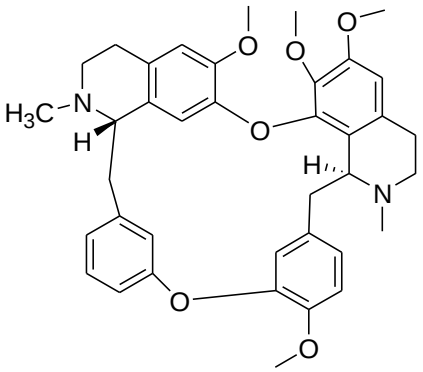
Hayatidine (**234**)



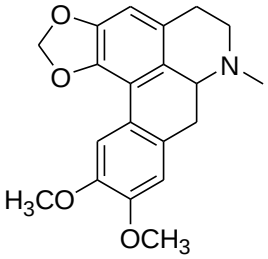
Cissampareine (**235**)



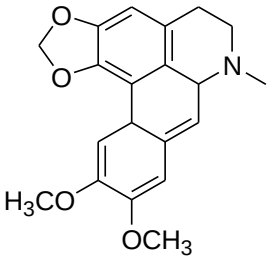
Cyclanoline (**236**)



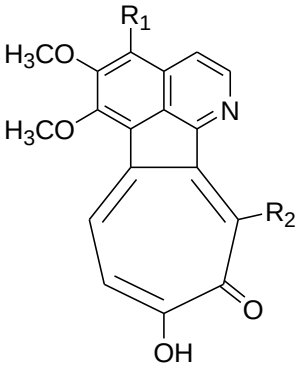
(+)-tetrandrine (**237**)



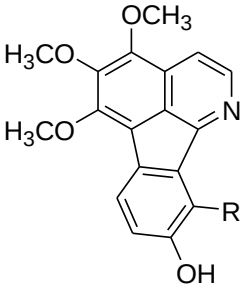
Dicentrine (**238**)



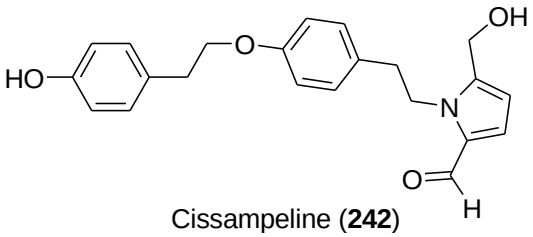
Dehydrodicentrine (**239**)



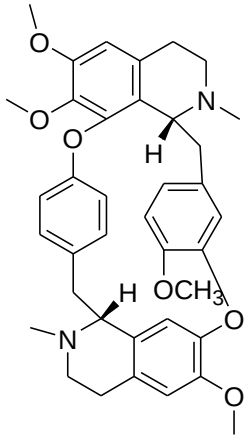
Grandirubrine (**240**) R1= OCH_3, R2 = H
Pareirubrine A (**245**) R1= R2 = OCH_3
Pareirubrine B (**246**) R1 = R2 = H



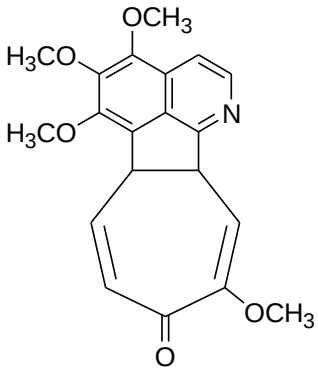
Norimeluteine (**241**): R = OCH_3
Norruffscine (**248**): R = H



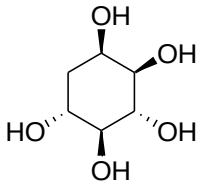
Cissampeline (**242**)



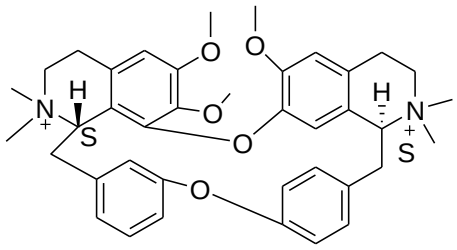
Insularine (**243**)



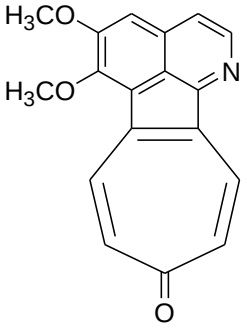
Isoimerubrine (**244**)



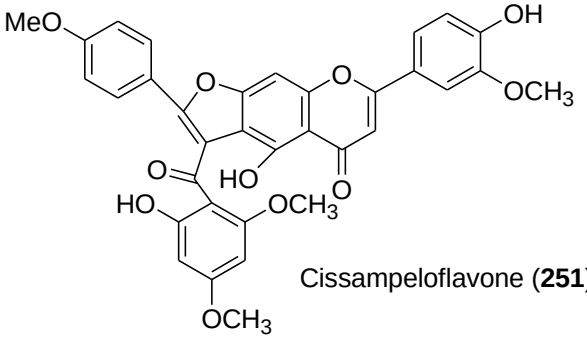
D-querticol (**247**)



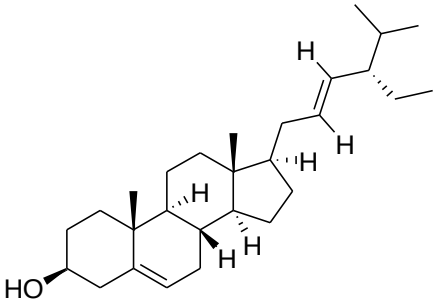
Dimethyltetrandrinium (**249**)



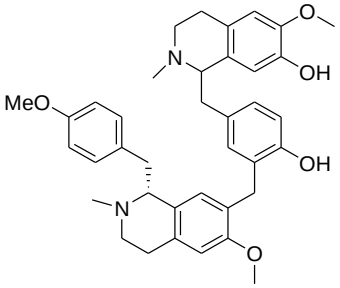
Pareitropone (**250**)



Cissampeloflavone (**251**)



(-)-Stigmasterol (**252**)



Isoliensinine (**253**)

Figure 2.20: Chemical compounds isolated from *Cissampelos*.

2.6.2 *Prosopis juliflora* (Mesquite tree)

Prosopis juliflora (SW.) DC (Fabaceae), commonly known as mesquite tree and locally as *Mathenge* is generally described as a hardy evergreen thorny xerophyte characterized by fast growth rates, adaptively in poorly drained alkaline soils, allelopathic and with drought resistance potentials (Muthana & Arora, 1983). The plant grows to a height of around 12 m with bi-pinnate light to dark green compound leaves and 5-10 cm long non-scented green to yellow flowers clustered around the ends of branches. The pods borne by this plant are ~ 25 cm long with an average of 15-30 seeds per pod. *P. juliflora* is native to Central and South America but due to introductions for its initial well-thought benefits it has widely spread to other parts of the world. Of the known 44 species, some of its closely related species within the *Prosopis* genera are *P. grandulosa*, *P. africana*, *P. angulate*, *P. cineraria*, *P. chilensis*, *P. flexusa*, *P. tamarugo*, *P. alpha*, *P. alba*, *P. nigra*, *P. pallida*, *P. alpataco*, *P. denudans*. In the early 1980s, the mesquite plant was introduced in Kenya to mitigate deforestation and provide a permanent solution to wood and fodder shortages. However, the plant has been alarmingly invasive, threatening loss of productive land and negatively impacting livelihoods of the local residents in Baringo (Mwangi & Swallow, 2005). A similar scenario has been witnessed in Mali, Tanzania, Chad, South Africa, Niger, India, Sudan, Ethiopia and other regions where the plant grows (Pasiecznik, 1999). Recently, it was reported that the thickets and flowers of the invasive plant *P. juliflora* support malaria transmission by promoting survival of female *Anopheles* mosquitoes in Malian villages (Muller et al., 2017a), joining the list of

reported malaria-supporting invasive plants such as *Acacia salicina* (Fabaceae), *Eucalyptus cladocalyx* (Myrtaceae), *Senna didymobotrya* (Fabaceae), *Tecoma stans* (Bignoniaceae) and *P. hysterophorus* (Asteraceae) (Nyasembe *et al*., 2015; Stone *et al*., 2018). Although *P. juliflora* was introduced to provide fodder to animals, intoxication exerted by its piperidine alkaloids, juliprosopine (**87**) and juliprosine (**254**; Figure 2.21), has been reported to affect cattle and goats. This intoxication is mediated through mitochondrial damage and cytoplasmic vacuolation in glial cells and neurons (Silva et al., 2013).

Prosopis juliflora has been documented as a folk remedy against scorpion stings, snake bites, rheumatism, cancer, diarrhea, stomach aches, sore throat, measles, flu, eye infections, skin lesions, head cold, wounds, dysentery, and inflammatory conditions (Henciya *et al*., 2017). Experimental investigations of *P. juliflora* extracts have reported potent antimicrobial, molluscicidal, antiprotozoal, antioxidant, anti-emetic, antihelmintic, mosquitocidal and anticancer activities. The bioactivity of these extracts is strongly linked to piperidine alkaloids – julifloridine (**255**), juliprosopine (**87**), julifloricine (**256**), juliflorinine (**257**), and juliprosinene (**258**) (Ahmad *et al*., 1989), and flavonoids although other compounds have been implicated in mediating significant biological activities (Prabha *et al*., 2014). The unusual amounts of flavonoid (-)-mesquitol (**259**) contributes to the plant's antioxidant properties (Sirmah *et a*l., 2009). An investigation by Al-Musayeib *et al*., (2012) reported that methanolic extracts of Saudi Arabian *P. juliflora* fruits displayed antimalarial (IC_{50} 4.1 µg/mL, SI 12.2 against K1) and trypanosomicidal activity against *T. cruzi* (IC_{50} 10.4 µg/mL) and *T. brucei* (IC_{50} 2.0 µg/mL, SI 24.9). In contrast, hydroethanolic extract of Iranian *P. juliflora* elicited moderate activity at IC_{50} 14.78 and 4.68 µg/mL against K1 and CY27, respectively, but its dichloromethane fraction exerted high activity against CY27 (IC_{50} 1.4 µg/mL) and K1 (IC_{50} 9.95 µg/mL)(Ramazani et al., 2010). Elsewhere, motivated by the findings of Al-Musayeib et al. (2012), *in vivo* studies by Batista *et al*., (2018) in Brazil demonstrated that an alkaloid-rich fraction containing julifloridine (**255**), juliprosopine (**87**) and alkaloid-enriched extracts suppressed *P. berghei* NK65. In that study, **255** (*in vitro* IC_{50}

35.1 µM against *P. falciparum* W2) caused significant *P. berghei* parasitemia inhibition (15–35%) at 2 mg/kg compared to 50 mg/kg of CQ and great survival of the infected mice but 58-fold lower than **87**. Lima *et al*., (2017) investigated the bioactivity of *P. juliflora* pods against goat gastrointestinal helminthes. The findings demonstrated that the alkaloid-rich fraction containing **87** had high ovicidal activity (IC_{50} 1.1 mg/mL), but highly toxic to Vero cell lines. In another study, the antiplasmodial activity of juliprospine **87** (IC_{50} 604 nM) was postulated to be exerted through interaction with the inner mitochondrial membrane, uncoupling generation of ATP (Maioli et al., 2012). Garbi *et al*., (2014) reported antigirdial and amoebicidal activities of *P. juliflora* petroleum ether leaf extracts eliciting 71.97% mortalities while larvicidal activities have been displayed by the acetonic leaf extracts against *An. stephensi* (Senthilkumar et al., 2009).

The major alkaloid juliprosopine non-competitively inhibits acetyl cholinesterase activity (IC_{50} 0.42 µM) affording dose-dependent anti-Alzheimer's therapeutic effects (Choudhary et al., 2005). Antimicrobial activities of this plant have been attributed to juliprosopine (**87**), isojuliprosine (**260**), **258**, piperidine alkaloid *N*-β-chloropropionyltryptamine (**261**), myoinositol-4C-methyl (**262**) (Ahmad *et al*., 1986; Aqeel *et al*., 1989; dos Santos *et al*., 2013). The flavonoid, patulitrin (**263**) from the flowers and leaves induce antiproliferative activities against cancerous cells by arresting mitotic divisions and provoking chromosome aberrations (Sathiya & Muthuchelian, 2010). Tannins from the plant leaves have been associated with antihelminthic activities against gastrointestinal nematodes in sheep (Mutembei et al., 2015).

Other compounds identified from *P. juliflora* include; flavanones isolated from the ethanolic extract of roots include 3′,4′-dihydroxy 5-methoxy 6-methyl flavanone 7-*O*-β-D-glucopyranoside (**264**) and 7,4′-dimethoxy 6,8-dimethyl flavanone 5-*O*-β-D-galactopyranoside (**265**) (Malhotra & Misra, 1983). A terpenoid diketone, prosopidione (**266**), was isolated from the leaves (Ahmad & Sultana, 1989).

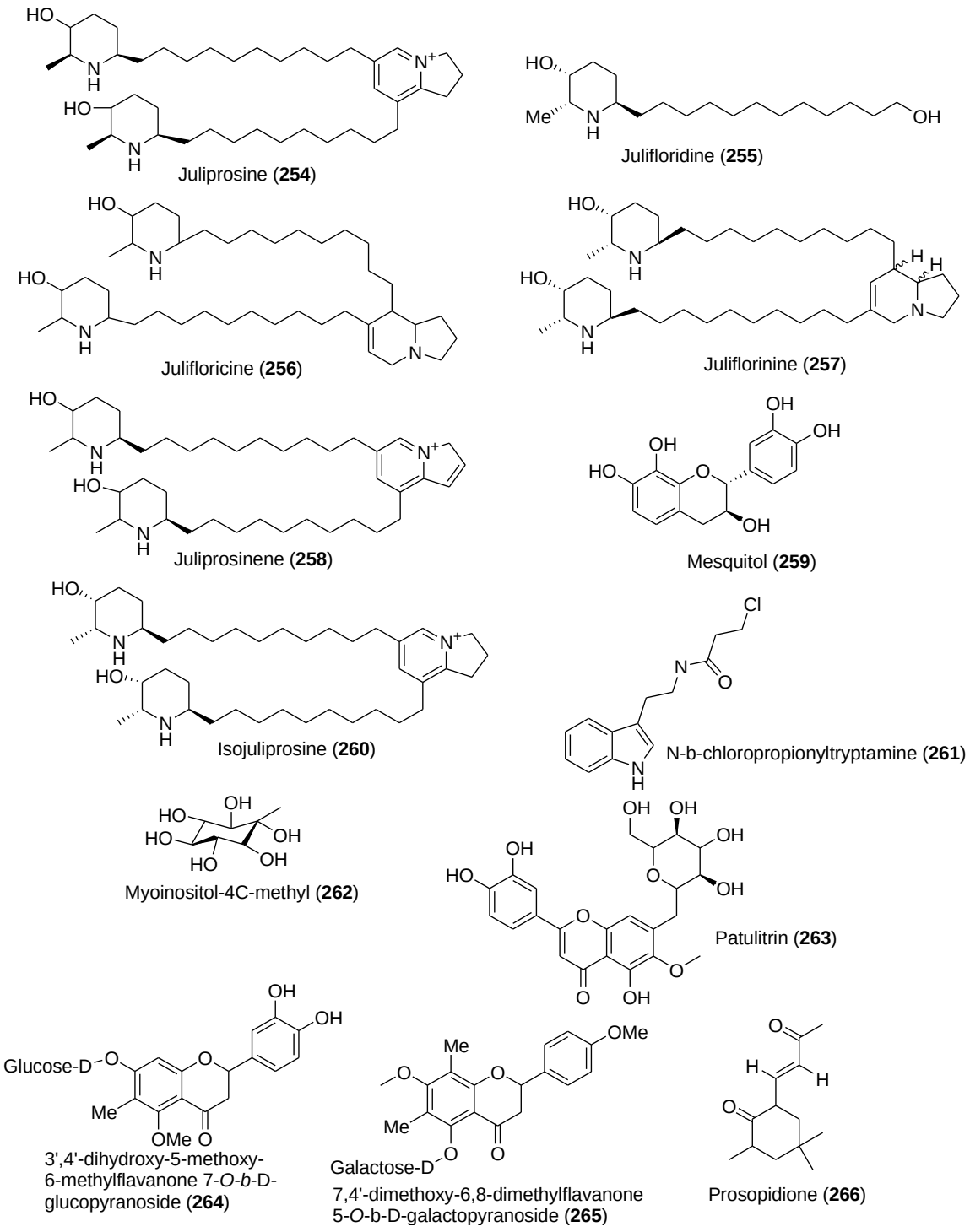


Figure 2.21: Chemical compounds isolated from *P. juliflora* plant parts.

2.6.3 *Zanthoxylum chalybeum* (Knobwood)

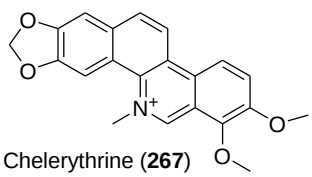
Z. chalybeum (Rutaceae) also known as *Knobwood* in English, *Mukenea/Kikenea* among the Kamba community, and *Mjafari* in Swahili, is among the 35 species of the largest genera (*Zanthoxylum*) of plants in Africa. The tropical plant is native to Burundi, Ethiopia, Kenya, Lesotho, Mozambique, Tanzania, Uganda, Namibia, Zambia, Swaziland, Somalia, South Africa, Democratic Republic of Congo, Malawi, and Zimbabwe. *Z. chalybeum* is botanically characterized by a height of ~10-12 m, knobbed thorny and fairly white stripped pale greyish stem, strongly scented yellowish green flowers and deciduous spiked 6-22 cm long leaves that leave a sweet sensation when chewed and used to prepare wild tea-like beverages and medicinal decoctions (Orwa et al., 2009). Depending on the ecosystem, the fruiting season of *Z. chalybeum* varies with Kenyan plants bearing fruits in March or July-August yearly.

Documented ethnopharmacological potentials tied to this plant include; appetite improvement for children among the Maasai and Sonjo communities by adding bark juice into milk, anti-inflammatory and antibacterial activities (Matu & van Staden, 2003). Additionally, the roots have been used for antimalarial, treatment of colds and coughs, sores, wounds, relieving tooth aches and headaches among the south coast, Msambweni residents (Nguta et al., 2010). Hot infusion or decoction prepared from the stem barks was documented being used among the Kamba community in management of diabetes mellitus (Keter & Mutiso, 2012). Furthermore, traditional healers in Dar es

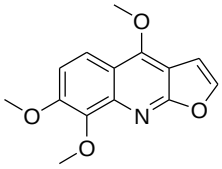
Salaam, Tanzania administer orally three spoonfuls of root bark decoction three times a day for treatment of epilepsy (Moshi et al., 2005). In another study, *Z. chalybeum* was documented among the plants used by Ugandan traditional medicinal practitioners to treat tuberculosis (Tabuti et al., 2010). In some African communities, the root and stem bark decoctions have been used for managing sickle cell disease, snake bites, hernia, rheumatism, yellow fever, body pains, elephantiasis, diabetes, abdominal pains, tuberculosis, asthma, intestinal worms, dysmenorrhea, treating fever, bubonic plague, measles, toothaches, and sexual impotence. Infusions from roots and leaves are drunk to treat oedema, convulsions, body pains, psychiatric problems, stomach pains, uterine fibroids, female sterility, venereal diseases, ease child-birth process, and treatment of livestock anaplasmosis, fever, and intestinal tract infections (Engeu et al., 2008; Muganga et al., 2010; Nibret et al., 2010). Various biological activities including larvicidal, antimicrobial, cytotoxicity, trypanocidal, antimalarial, anti-inflammatory, antiviral, anticancer have been attributed to its isoquinoline alkaloids, steroids and phenolic derivatives.

A number of chemically active compounds have been isolated from *Z. chalybeum* plant parts. Krane et al., (1984) isolated and reported chelerythrine (**267**; Figure 2.22), a furoquinoline alkaloid skimmianine (**268**), and arnottianamide (**269**). Furthermore, rutaecarpine (**270)** and lignans were reported by Sheen and co-workers (Sheen et al., 1994, 1996). From the roots, Kato *et al*., (1996) isolated colored protoberberines jatrorrhizine (**271**) and palmatine (**272**), (+)-tembetarine (**273**), (+)-magnoflorine (**227**), a quinolone alkaloid (+)-*N*-methylplatydesmine (**274**), (-)-oblongine (**275**), chelerythrine (**267**), nitidine (**276**), and (-)-*cis*-*N*-methylcanadine (**277**). Aluoch, (2000) isolated benzophenanthridine alkaloids dihydrochelerythrine (**278**) and chelerythrine (**267**), 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane (**279**), lignan (-)-asarinin (**280**), a quinolone alkaloid 4-methoxy-*N*-methyl-quinolin-2-one (**281**) and triterpene lupeol (**282**) from the root barks. Rahman and co-investigators reported nitidine (**276**) and *N*-methylflindersine (**283**) (Rahman et al., 2005). A coniferyl alcohol derivative 2,3-epoxy-6,7-methylene dioxyconferyl alcohol (**284**) alongside dihydrochelerythrine (**278**) were isolated from

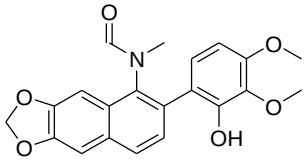
Ethiopian *Z. chalybeum* roots (Anza et al., 2014). From the stem bark, an antimalarial active compound fagaramide (**285**) with IC_{50} 2.8 µg/mL against CQ-resistant FCR3 but less active against CQ-sensitive *Plasmodium* NF54 strain (IC_{50} 16.6 µg/mL) was isolated (Adia et al., 2016). Similar bioactivity was reported by Gessler et al., (1994) who used the root barks. A GC-MS analysis conducted on essential oil extracted from mature and young leaves collected from Zimbabwe detected abundance of limonene (**286**) (48.06%), geranial (**287**) (13.21%), neral (**288**) (12.11%), terpinolene (**289**) (7.91%), linalylpropionate (**290**) (7.89%), citronellal (**291**) (7.30%), camphene (**292**) (5.28%), neryl acetate (**293**) (5.74%) and terpinen-4-ol (**294**) (5.28%) (Chagonda et al., 1994). Previous studies conducted on *Zanthoxylum* species have indicated availability of mosquitocidal compounds (Zhang et al., 2009; Overgaard et al., 2014; Moussavi et al., 2015; Kim & Ahn, 2017; Pavela & Govindarajan, 2017), but such information is lacking for *Z. chalybeum*.



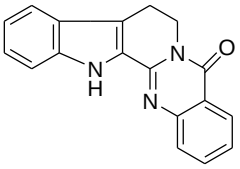
Chelerythrine (**267**)



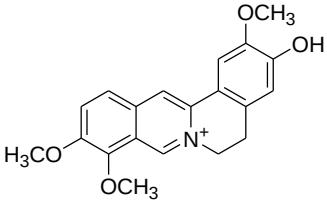
Skimmianine (**268**)



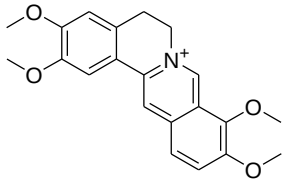
Arnottianamide (**269**)



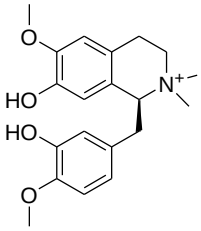
Rutaecarpine (**270**)



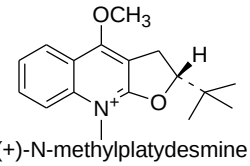
Jatrorrhizine (**271**)



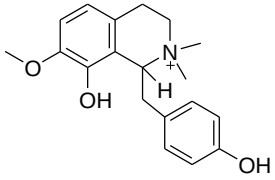
Palmatine (**272**)



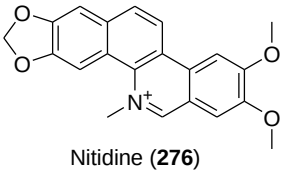
(+)-tembetarine (**273**)



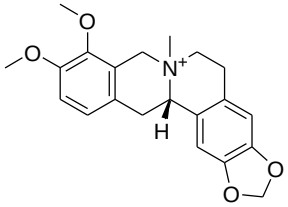
(+)-N-methylplatydesmine (**274**)



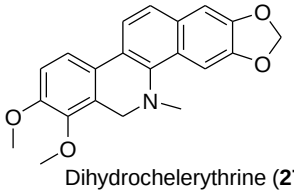
(-)-Oblongine (**275**)



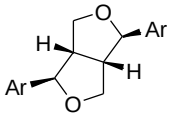
Nitidine (**276**)



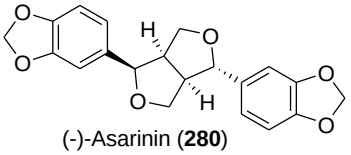
cis-*N*-Methylcanadine (**277**)



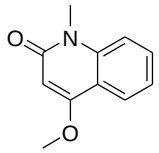
Dihydrochelerythrine (**278**)



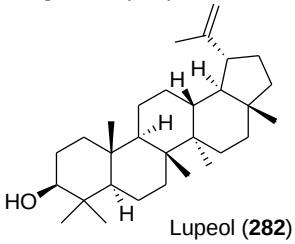
2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane (**279**)



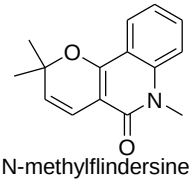
(-)-Asarinin (**280**)



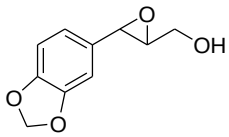
4-Methoxy-N-methylquinolin-2-one (**281**)



Lupeol (**282**)



N-methylflindersine (**283**)



2,3-epoxide-6,7-methylenedioxide coniferyl alcohol (**284**)

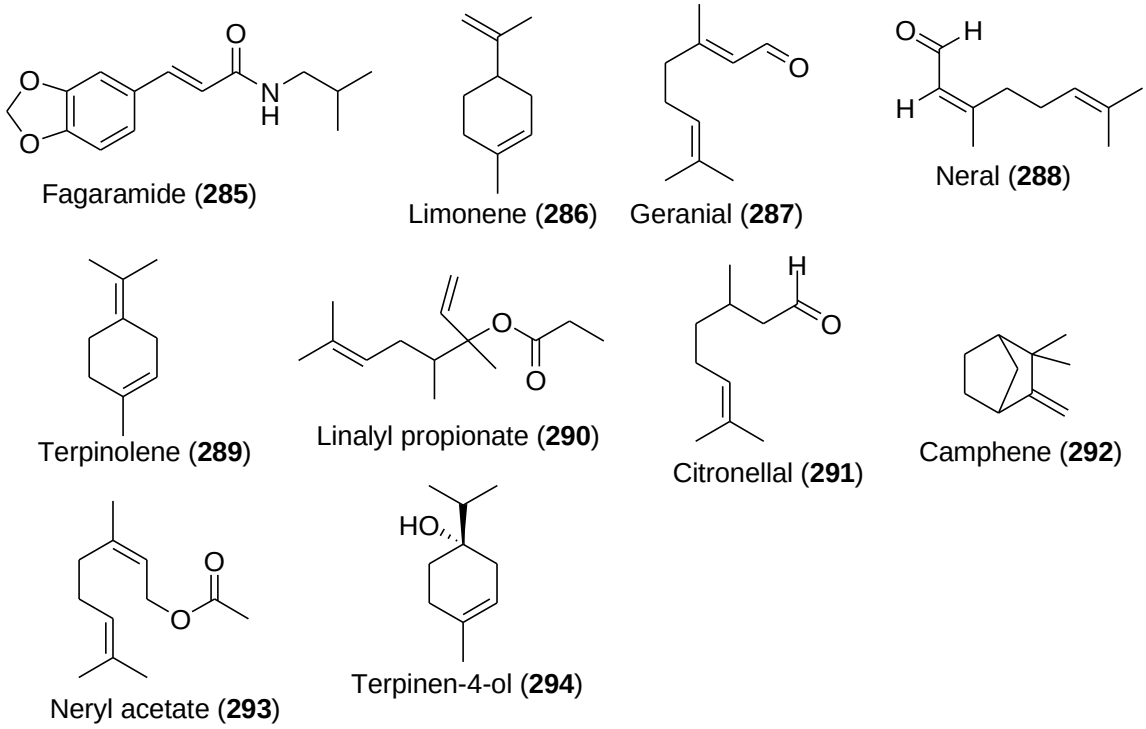


Figure 2.22 Chemical compounds isolated from *Z. chalybeum* plant parts

2.7 Natural compounds molecular targets and mechanism identification

Natural products largely target an intricate network of intracellular proteins to mediate their anti-infective and -cidal effects from unbiased phenotypic drug screening platforms. Delineation of these unknown targets as well as mechanisms deconvolution exerted by these compounds is quite a challenging task, owing to the complexity of treatment effects and cost implications of most approaches (Futamura et al., 2013; Li et al., 2021). Among the frequently used approaches are; chemical genetic characterization, -omics profiling, and chemical probe-based pull-down target identifications (Guiguemde et al., 2010; Lee & Bogyo, 2013; Siwo et al., 2015; Cowell et al., 2018; Yang et al., 2021). Besides these, induced phenotype-based characterization and *in silico* pathway enrichment from small-molecule public libraries such as ChEMBL, DrugBank, ChemBank can inexpensively identify mechanisms of action and molecular targets (Gamo et al., 2010; Hughes et al., 2021; Trapotsi et al., 2022). This study capitalized on

combined hypothesis-driven phenotypic and systems computational pathway analyses for characterization of treatment mechanisms of the bioactive compounds.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

Part of the experimental studies were carried out at the US Army Medical Research Directorate - Africa, Kenya (USAMRD-A/K), Kisian Field Station, Kisumu. *In vitro* antimalarial screenings were conducted at the Malaria Drug Resistance (MDR) laboratory, while the standard membrane feeding assays were conducted at the Entomology Department's Containment Unit. Extractions, isolations, and larvicidal assays were carried out at the International Centre of Insect Physiology and Ecology (*icipe*, Duduville Campus), Nairobi.

3.2 Plant material sampling

The experimental plant materials for this study were collected and processed in accordance with WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants (WHO, 2003). These plant samples were collected between mid-July and early August, 2018 from the sites tabulated in Table 3.1. A literature-based sampling strategy informed by the geographical sites of the plants was pursued.

Table 3.1: Description of sampling sites and plant material extractions.

Plant	Family	Part used	Sampling site	GPS coordinates	Extraction solvents	% yield
Murraya koenigii	*Rutaceae*	Leaves	Kibwezi	02˚23'13"S, 038˚00'03"E, 899 m asl	CH_2Cl_2/MeoH	7.91
Camellia sinensis	*Theaceae*	Leaves	Limuru	01˚07'10"S, 036˚39'37"E, 2225 m asl	70% Acetone	25.45
Agerantum conyzoides	*Asteraceae*	Aerial shoots	JKUAT main campus	01˚05'56.3"S, 037˚00'51.3"E, 1525 m asl	CH_2Cl_2/MeOH	3.04
Schinus terebenthifolius	*Anacardiaceae*	Leaves	*icipe* campus	01˚13'17.8"S, 036˚53'47.2"E, 1603 m asl	CH_2Cl_2/MeOH	3.53
Prosopis juliflora	*Fabaceae*	Leaves	Marigat town	00˚28'25.6"N, 035˚58'49.5"E, 1042 m asl	CH_2Cl_2/MeoH	14.65

Mangifera indica	*Anacardiaceae*	Stem bark	Mathemba, Makueni	01˚53'27.9"S, 037˚45'06.7"E, 1133 m asl	95% Ethanol	8.58
Zanthoxylum chalybeum	*Rutaceae*	Root bark	Mathemba, Makueni	01˚53'22.1"S, 037˚45'11.9"E, 1109 m asl	CH_2Cl_2/MeoH	5.55
Vitex payos	*Verbenaceae*	Root bark	Mathemba, Makueni	01˚53'32.1"S, 037˚45'33.0"E, 1086 m asl	Acetone	11.16
Terminalia brownie	*Combretaceae*	Stem bark	Mathemba, Makueni	01˚53'22.7"S, 037˚45'13.9"E, 1111 m asl	CH_2Cl_2/MeOH	17.76
Persea Americana	*Lauraceae*	Seeds	Kandara, Murang'a	00˚57'46.0"S, 037˚05'26.4"E, 1536 m asl	70% Acetone	3.52
Chrysanthemum cinerariifolium	*Compositae*	Flowers	Kinungi East, Naivasha	00˚45'30.9"S, 036˚30'41.5"E, 2318 m asl	*n*-Hexane	2.10
Cissampelos pariera	*Menispermaceae*	Roots	Kiritiri, Embu	00˚42'54.6"S, 037˚38'28.1"E, 1561 m asl	CH_2Cl_2/MeOH	18.39
Vitex schiliebenii	*Verbenaceae*	Leaves	Near Gede town, Malindi	03˚18'08.3"S, 039˚59'51.8"E, 34 m asl	Acetone	2.31

Natural ecosystems in which medicinal plants grow greatly influence the regio- and stereochemical characteristics and bioactivity of the resultant phytoconstituents hence comprehensive descriptions of the sampling sites is paramount. *M. koenigii,* collected from Kibwezi, grew on a relatively flat land covered by deep red sandy alluvium soil. Kibwezi is approximately 192 Km south of Nairobi along Nairobi-Mombasa highway and experiences hot and dry climatic conditions characterized by bimodal rainfall pattern. Long rains fall during March/May and short rains in November/December every year amounting to ~ 300 mm. The semi-arid rangeland is dominated by commiphora and acacia vegetation cover (Gachimbi, 1990). These typical climatic conditions are also present in Mathemba village, Makueni where *M. indica, Z. chalybeum, T. brownii,* and *V. payos* plant materials were collected.

Prosopis juliflora (SW.) DC collected from Marigat in Baringo County grows alongside *Balanites aegyptiaca, Acacia mellifera*, cactus, and commiphora bushes forming dense impenetratable thickets. Marigat is approximately 250 Km north of Nairobi with Lake Baringo to the North and Lake Bogoria to the East. The low-lying semi-arid rangeland is characterized by high temperatures (> 32˚C) and relatively low annual rainfall ranging between 300 – 700 mm that fall from April to July (long rains) and between October and December (short rains) yearly. The dominant soils in this region are mainly poor drained clay loam with alluvial deposits derived from tertiary and/or quaternary volcanic and pyroclastic rock sediments weathered and eroded from Tugen highlands (Amadi et al., 2018). The Brazilian pepper (*S. terebenthifolius*) leaves were collected from a planted tree at *icipe*, duduville campus, Nairobi. *icipe* assumes the climatic conditions of Nairobi with an average annual rainfall of ~ 900 mm that falls between October-December (short rains) and long rains occurring in March-May. The average temperature is 19°C.

Fresh tea leaves (clone TRFK 6/8) were purchased from Limuru Archdiocesan farm in Limuru sub-county of Kiambu County ~30 Km northwest of Nairobi (Muema et al., 2016). This tea growing region is characterized by well drained, fertile acidic volcanic red soils derived from weathered volcanic rocks and experiences warm and temperate climatic conditions, a high annual rainfall of about 1200 mm from the bimodal-type of rain pattern and mean temperature range of between 10-25°C. The long rains fall between March and May while the short rains occur between October and November every year. The average relative humidity ranges from 54% in the dry months and 300% in the wet months of March-August. Similar climatic conditions are experienced at JKUAT main campus, Juja, the location *A. conyzoides* was collected, however this region receives relatively lower rainfall ~ 600 mm and has poorly drained shallow soils. Kinungi East in Naivasha sub-county is the region pyrethrum flowers were purchased. The region lies on the escarpment of the Great Rift Valley between the Kinangop plateau and the slopes of Nyandarua Mountains adjacent to Naivasha town and Lake Naivasha. The region experiences the long rains between April-June and the short rains fall during September-October months reaching mean annual level of > 2000 mm, relatively high

diurnal temperature of ~ 28˚C and is covered by fertile and permeable weathered volcanic soils that favor horticultural farming (http://www.nyandarua.go.ke/). Pyrethrum farming is a dominant economic venture in this region although fluctuating low prices have threatened the practice promoting crop uprooting and abandonment.

Vitex schiliebenii, collected from Gede region in Malindi sub-County, grew on the flat coastal plain with the Magarini red sandy soil adjacent to the famous indigenous Arabuko-Sokoke rain forest off the Kenyan coast (Nyamoita et al., 2013). Malindi experiences long rains from April to June and short rains during October-November with mean annual rainfall ranging between 750 and 1200 mm. A semi-arid climatic condition prevails within this region with mean daily temperature of 22-30˚C and average relative humidity of 65%. Avocado seeds used in this study were collected from Kandara, Murang'a County. Generally, the region experiences an equatorial type of climate characterized by variable annual rainfall ranging between 1400-2000 mm with bimodal pattern. Long rains fall in March-May while the short rains occur during October-December. The mean annual temperature ranges between 18-21˚C. The soils are deep, well drained, weathered humic nitisols (red Kikuyu loams) that support several food crops including *P. americana* under this study due to the influence of the Aberdares and Mt. Kenya. *C. pariera,* collected from Kiritiri in Mbeere South sub-County, Embu County grew in a semi-arid region that covers the lower south eastern slopes of Mt. Kenya near Mwea National Reserve and adjacent to the Kamburu and Masinga dams of the Seven Tana River Forks Hydro-electric Scheme. The region receives a bimodal rainfall pattern with long rains falling between April and May and short rains occurring in November-December amounting between 640-2000 mm. Annual temperatures range between 17-30˚C. The soils are well drained, deep red loam of Lithosol/Canibisol soil types (Gachimbi, 2002).

3.3 Plant material processing

The collected plant materials were taxonomically authenticated by Mr. Patrick Chalo B. Mutiso of University of Nairobi Herbarium where all the voucher specimens were

deposited for future reference. The assigned sample voucher numbers were: *S. terebenthifolius* (JMM2018/01), *C. cinerariifolium* (JMM2018/02), *M. indica* (JMM2018/03), *V. payos* (JMM2018/04), *V. schiliebenii* (JMM2018/05), *Z. chalybeum* (JMM2018/06), *P. americana* (JMM2018/07), *P. juliflora* (JMM2018/08), *T. brownii* (JMM2018/09), *C. pariera* (JMM2018/10), *M. koenigii* (JMM2018/11), *A. conyzoides* (JMM2018/12), and *C. sinensis* (JMM2018/13). All the plant materials were shade-dried at 25 ± 2˚C and milled into fine powder using an electric grinder (Retsch SM 100, Germany) ready for extractions.

3.4 Preparation of plant extracts

All the solvents used for plant material extractions were of analytical grade purchased from either Fisher Scientific (Loughborough, UK) or Sigma-Aldrich (St. Louis, Mo, USA) unless otherwise stated.

With exception of *C. sinensis*, *V. payos*, *V. schiliebenii*, *P. americana*, *M. indica*, and *C. cinerariifolium*, the powdery materials of other 7 plant samples – *M. koenigii* (318.84 g), *A. conyzoides* (330.24 g), *S. terebinthifolius* (821.37 g), *P. juliflora* (495.87 g), *Z. chalybeum* (815.71 g), *T. brownii* (343.62 g) and *C. pariera* (334.78 g) - were separately extracted with dichloromethane (CH_2Cl_2) / methanol (MeOH) (1:1 (*v/v*), 2 × 1.5 L) (Table 3.1). *C. sinensis* (256.52 g) and *P. americana* (604.98 g) were extracted with 70% acetone (2 × 1.5 L) respectively, while *V. payos* (112.76 g) and *V. schiliebenii* (634.34 g) were extracted with 2 × 1.5 L acetone, respectively (Nyamoita, 2013). Stem bark powder of *M. indica* (601.62 g) was extracted with 95% ethanol as described by Ghosh *et al*., (2012). Crude extract of *C. cinerariifolium* (10.52 g) was prepared as described (Hata et al., 2011) by soaking 2-week old mature flower powder (500.99 g) in *n*-hexane (2 × 1.5 L). Defatting of all the plant material was performed with *n*-hexane prior to extractions except for *C. cinerariifolium*. All the extracts were filtered through a glass filter and concentrated under low pressure rotary evaporator (Laborota 4000 efficient, Heidolph, Germany). The resultant extracts were stored at -20˚C until use for antimalarial screening.

3.5 Isolation and identification of bioactive compounds

3.5.1 Malaria SYBR Green I-based fluorescence assays

Preliminary antimalarial screening of the crude extracts against asexual blood stages of multi-drug resistant (CQ/PYR) PfW2 strain and a human clinical isolate (MDH 0038), using an established malaria SYBR Green I-based fluorescence assay (Akala et al., 2011). This assay is based on parasite DNA content as a measure of *Plasmodium* growth and subsequent inhibition by antimalarial compounds. 100 µL aliquots of highly parasitemic cultures were reconstituted at 1% parasitemia and 2% hematocrit and dispensed into compound pre-dosed 96-well plates, for 72-h incubation at 37˚C. Final DMSO concentration in all assays did not exceed 0.2% *v/v*. Replication inhibition activity was analyzed in 3 replicates by SYBR Green I-based readouts upon parasitized RBCs lysis using SYBR green lysis buffer that exposes and intercalates parasite DNA. Following an overnight incubation of the drug plates in dark at room temperature, the relative fluorescent readouts were acquired using a Tecan Genios Plus microplate reader at 485 nm excitation and 535 nm emission wavelengths. Immediate *ex vivo* (IEV) susceptibility assays were performed against *P. falciparum* clinical isolates (0-6 h post-phlebotomy collection; at parasitemia level ≥1%) collected from consenting individuals with uncomplicated malaria at Kisumu and Kombewa study site clinics (Approved protocols; KEMRI SSC # 1330 and WRAIR # 1384). Following an adjustment to 1% parasitemia, these freshly collected parasites were directly tested for their susceptibility to the plant compounds without prior culture-adaptation, alongside a panel of standard antimalarial drugs.

To investigate the speed- and stage-of-action of the plant compounds against asexuals, synchronized Dd2 rings (0-5 h post-invasion (hpi); > 98%), respectively, were examined at 1% parasitemia against DMSO-treated parasites at different treatment periods within the 48-h intraerythrocytic replication; 5-16, 17-29, and 29-41 hpi. SYBR Green I IC_{50} speed assay was adopted for time specificity of isoliensinine action within the standard

72-h analysis. After each treatment period, stage-specific parasite morphological analyses and imaging of Giemsa-stained thin films were performed.

3.5.2 Bioactivity-guided fractionation, TLC chromatography, LC/MS and GC/MS analyses

Active plant extracts from screening assays were fractionated using column chromatography. ~ 40 g of *C. pariera* root extract were adsorbed in silica gel (Kiesegel 60 M, [0.004-0.063 mm mesh size], Macherey-Nagel GmbH & Co.KG, Düren, Germany] and fractionated on silica-packed column through a gradient elution of *n*-hexane-EtOAc (1:0, 9:1, 7:3, 1:1, 3:7, 1:9, 0:1) then EtOAc-MeOH (1:1, 1:9, and 0:1) yielding forty 100-mL fractions. Fractionation of *P. juliflora* biomass (42.55 g) was carried out under an increasing gradient elution of CH_2Cl_2-EtOAc (1:0, 7:3, 1:1, 3:7, 1:9, 0:1) and finally 100% MeOH affording 24 fractions. For *Z. chalybeum*, 49 g of the brown-yellow oily extract was adsorbed in silica gel (Kiesegel 60 M [0.004-0.063 mm mesh size]) and loaded on a silica gel-packed column. Fractionation was performed through an increasing gradient solvent system of EtOAc in *n*-hexane (1:0, 9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9, 0:1) and finally 100% MeOH yielding 23 100-mL fractions.

Thin layer chromatography (TLC) profiling of the resultant fractions was developed by iodine staining with various solvent systems for the three plant extracts. Using *n*-hexane:EtOAc:MeOH (1:2:1) solvent system for *C. pareira* and *Z. chalybeum*, the separation patterns analysis yielded 8 and 12 pooled fractions; CP01-CP08 and ZCFr.1-12, respectively. For *P. juliflora*, a CH_2Cl_2:EtOAc:MeOH (1:1:2) solvent system was used and the analysis of fractions resulted in 10 major fractions; PJ01 - PJ10. The resultant fractions were rotor evaporated to ~ 0.5 mL, air-dried, and stored at -20˚C until screened for their bioactivity.

Two highly active fractions based on antimalarial potency against clinical isolates and CQ-sensitive D6 strain, CP08 (eluted with 1:9-0:1 EtOAc:MeOH) from *C. pariera* and PJ10 (water soluble fraction) from *P. juliflora* were prioritized for analysis. PJ10 had a single spot based on TLC, and was not purified further giving juliprosopine (**87**), but

CP08 was re-chromatographed over silica gel with MeOH/H_2O, 13:7, 7:13, 0:1 to yield antimalarial compound isoliensinine (**253**). The isolates were subjected to LC-ESIMS/MS for analysis. Approximately 1 mg of each isolate was dissolved in CHROMASOLV® LC-grade MeOH/H_2O (1:1 *v/v*) and centrifuged at 13000 rpm for 5 min after which 1.0 mL of the supernatants were separately transferred into autosampler HPLC vials. Chromatographic separation of injected 5 µL analytes was achieved on Agilent's 1290 Infinity ultra high performance liquid chromatography (UHPLC) series fitted with a rapid resolution reverse phase C_{18} column (1.8 µm, 2.1 × 150 mm i.d; Agilent Technologies). The column temperature was maintained at 45˚C. At a flow rate of 0.4 mL/min, an elution gradient of 95:5 (0-3.5 min), 50:50 (3.5-17 min), 0:100 (17-20 min) and 95:5 (20.01-25 min) was carried out over 25-min run with a binary solvent system of H_2O (solvent A) and MeOH (solvent B). The UHPLC was interfaced with Agilent Technologies 6490 model Triple Quad mass spectrometer equipped with iFunnel JetStream electronspray source operating in the positive ionization mode using dynamic multi-reaction monitoring (MRM) software features. ESI settings were adjusted at gas temperature 200˚C, gas flow 11.0 L/min, capillary voltage 3363 V, nebulizer 30 psi, sheath gas flow 8.0 L/min, and sheath gas temperature 375˚C. Nitrogen was used as both the nebulizer and collision gas.

Full mass spectra scans from the range of *m/z* 100 to 1000 Da were acquired being controlled by Agilent MassHunter Qualitative Analysis software version B.06.00. Structural identification and characterization of the resultant molecular ions was performed by comparing published literature data and chemical databases (METLIN, ChemSpider, PubChem, ZINC15, ChEMBL).

To identify the chemical entities eliciting the toxicity effects in *n*-hexane-soluble fraction (ZCFr.5) against mosquito larvae, a GC/MS–TQ8040 system (Shimadzu Corp., Kyoto, Japan) equipped with a mass selective detector and fitted with a silica capillary column (SH-Rxi-5HT; 30 m × 0.25 mm internal diameter × 0.25 µm film thickness) was used for analysis. Ultra-pure grade helium at a flow rate of 1.44 mL/min was used as the

carrier gas. The injector temperature was maintained at 280°C, and a sample volume of 1 µL injected. The initial oven temperature was programmed at 80°C for 5 min then ramped at rates of 15°C/min to 280°C (held for 8 min). For data acquisition, the GC-MS was operated in the full scan mode with Shimadzu's GC-MS Smart Pesticide Database (version 1) used as a foundation of analysis and identification of analytes.

3.6 Parasites culturing

Asexual *P. falciparum* intraerythrocytic parasites (D6, W2, Dd2 clones, and human clinical isolates) were cultured at Malaria Drug Resistance (MDR) laboratory of USAMRD-A/K, Kisian Field Station (under approved protocols WRAIR #1919 and KEMRI SSC #3126). Briefly, parasitized erythrocytes at 0.5% parasitemia in 4% haematocrit (O^+ human blood) were cultured at 37ºC with 90% N_2, 5% CO_2 and 5% O_2 in RPMI 1640 medium (Gibco Life Technologies) supplemented with 20% heat-inactivated ABO human serum, 5.94 g/L HEPES (Sigma-Aldrich), 2 g/L glucose, 2 mM L-glutamine, 4 µg/mL hypoxanthine, 2 g/L NaHCO3 (Sigma-Aldrich), and 10 µg/mL gentamicin sulfate (Gibco Life Technologies). To generate highly synchronous cultures, 5% (w/v) D-sorbitol treatments at the ring stage for 10 min at 37˚C were performed. Spent medium was aseptically replaced every two days until attainment of peak parasitemia level (5-8% rings) for antimalarial testing.

Highly productive gametocyte cultures were initiated from culture-adapted and 5% sorbitol-synchronized wild-type *P. falciparum* NF54 ring stage parasites and gametocyte-producing human clinical isolates. These asexual parasites were cultured at 1% parasitemia and 6% hematocrit in 20% complete RPMI 1640 medium (supplemented with 2 g/L glucose (Sigma-Aldrich, USA), 25 mM HEPES (Sigma-Aldrich, USA), 10 mL heat-inactivated ABO human serum, 25 mM $NaHCO_3$ (Sigma-Aldrich, USA), 5 µg/mL gentamicin (Life technologies, China), 100 µg/mL hypoxanthine (Sigma-Aldrich, USA), 0.5 g/L L-glutamine (Sigma-Aldrich, USA)). The culture flasks at final volume of 12.5 mL were incubated at 37˚C under controlled atmospheric gas mixture of 90% N_2, 5% CO_2 and 5% O_2 with medium change performed after every two days. No fresh

erythrocytes were added into the culture flasks post-induction; but the hematocrit was reduced with complete medium serum (CMS) to 3% on day 4 to induce nutritional stress required for gametocytogenesis. Gametocytes production from day 7 to 14 was routinely monitored by Giemsa-stained thin smears. Enrichment of the gametocyte cultures was performed by 65% percoll-PBS density gradient centrifugation conditioned at 37˚C.

3.7 *In vitro* gametocytocidal activity screening of pure compounds

For these analyses, 1× IC_{50} - 20× IC_{50} concentrations (obtained from asexual screening) were considered for evaluations against various stages of *Plasmodium* gametocytes. Firstly, the effect of the test materials on gametocyte production from 5% sorbitol synchronized ring stages was assessed as previously described (Jennison et al., 2019) with few modifications. Field isolates/NF54 parasite cultures at 1% parasitemia and 6% hematocrit were initiated in T25 cm^2 flasks until day 4, during which peak parasitemia levels had been attained (> 6%), when the hematocrit was reduced to 3% with 125% fresh CMS containing appropriate concentrations of the plant materials (solubilized in DMSO), or 0.2% DMSO vehicle. The spent medium was replaced daily at 37˚C for 12 days with Giemsa-stained blood smears prepared from each flask on days 5 and 7. For wash-out experiments, daily dosing was conducted for 72-h post-induction after which fresh 20% CMS compounds-free was used in daily feeds for the rest of gametocytogenesis period. Gametocytemia and morphology was monitored by counting a total of 3000 RBCs at oil immersion magnification for 3 independent experimental replicates.

To determine the gametocytocidal activity of the plant compounds, aliquots of 450 µL enriched gametocyte suspension at mature late-stages (day 14, ≥90% stage IV/V) diluted to 5% gametocytemia and 3% hematocrit were added into pre-dosed 24-well plates containing 50 µL of the compounds. The plates were incubated under standard culture conditions for 72-h in three independent sets of experiments. PQ was included as positive controls. At the end of each time-point incubation, excess supernatant was

carefully removed from each well and Giemsa-stained thin blood smears prepared for microscopy quantification of gametocytemia levels.

3.8 Molecular targets and ADME predictions

To gain insights into the molecular targets and functional pathway analyses of antimalarial action affected by the two compounds; juliprosopine (**87**) and isoliensinine (**253**), the manually curated ChEMBL compounds database (http://www.ebi.ac.uk/chembl) was queried at 70-90% confidence predictions. The lists of protein targets emerging from the searches were subsequently sorted from the "active" labelled. Subsequently, using the ChEMBL ID of each individual predicted target, the protein sequences in *.fasta* format were retrieved from UniProt database (http://www.uniprot.org) that were used to query for homologous *Plasmodium* proteins using *Blastp* in PlasmoDB, at default settings. SwissADME (http://www.swissadme.ch) was used to predict the ADME properties of the two antimalarials using default settings.

3.9 Mosquito colony maintenance

The *An. gambiae* mosquitoes (Kisumu strain) used in this study were raised from eggs obtained from USAMRD-A/K culture and/or Mbita strain and *Aedes aegypti* cultures maintained at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi. The eggs were seeded into plastic bowls (internal diameter = 85 cm) containing ~ 300 mL distilled water and internally lined with paper strips to increase hatching rates. The newly hatched larvae were fed on TetraMin®baby fish meal (Tetra GmbH, Melle, Germany) and on reaching 2nd instar, they were separately reared at densities of ~ 50 - 200 larvae per bowl. Rearing conditions were maintained at 30 ± 2°C, 67% relative humidity (RH) and 12 h light/dark cycles with rearing medium and food replenished every 2 days until pupariation. The pupae were collected into small plastic cups and placed in netted sterile standard metallic cages (30 × 30 × 30 cm) for eclosion. Newly emerged adults were separately maintained at laboratory-controlled conditions of 25°C, 70% RH and fed *ad libitum* on 6% glucose solution. Four days post emergence, the

females were provided with defibrinated bovine blood meal for 1 h to facilitate egg development for colony maintenance.

3.10 Assessment of juliprosopine (87) effects on mosquito fitness

Equal number of 6-h starved female *An. gambiae* mosquitoes (*n* = 80/per cage, 3 days old) of Kisumu strain were randomly aspirated for toxicity assessment of the potent antimalarial juliprosopine against a control group maintained on a 6% glucose meal supplemented with 0.1% DMSO in 1× PBS (pH 7.2). The prioritized juliprosopine was dissolved in 0.1% DMSO in 1× PBS (pH 7.2) and added into 6% glucose solution at final concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. All the formulated sugar meals were pre-warmed to 37°C and adsorbed into ~0.2 g cotton pads for direct feedings ad libitum. The dosed cotton pads were placed at the top of cage net and another at the base to maximize ingestions, and cotton pads replaced every 2 days. Mosquitoes were maintained in automated Forma™ environmental chambers (Thermo Fisher Scientific, USA) set at 80% humidity and temperature of 27˚C. Daily mortalities were recorded for 15 consecutive days (2 replicates), with cadavers removed from the individual cages.

Another cohort of 6-h starved and inseminated 200 females/cage was offered bovine bloodmeal imbibed with juliprosopine to final concentrations of 100 µg/mL, 200 µg/mL, and 400 µg/mL via artificial standard membrane feeder to assess effects on fecundity and subsequent hatchability. The control group was offered bloodmeal containing 0.1% DMSO in 1× PBS (pH 7.2). All the mosquitoes were fed for 1-h in dark mode and non-fed females aspirated off the cages and excluded from data analyses. In that regard, the cages were left with an average of 80 engorged females per treatment condition. Moist oviposition chambers were then provided for 48-h and the deposited eggs per cage counted under a light microscope for 1 gonotrophic cycle. All the experiments were replicated twice under insectarium conditions of 25 ± 1˚C and 70 ± 3% relative humidity in dark. To determine the viability of the deposited eggs from treated cohorts, the eggs were hatched in larval trays containing 100 mL tap water under larvae rearing conditions

of 30 ± 2˚C, 67% relative humidity (RH) and 12-h light/dark cycles, with 1st instar larvae from each tray counted for 3 days post-seeding.

3.11 Mosquito larvicidal bioassays for *Z. chalybeum*

Larvicidal bioassays were set up as outlined in the WHO guidelines (WHO, 2005) under controlled insectarium rearing conditions. Preliminary assay with ZCE at 25-500 ppm revealed 100% mosquito larval mortality within < 3 h, prompting lower dosages. Late third instar (L3) mosquito larvae (n = 25) were separately introduced into five beakers containing 100 mL of test solutions at different concentrations (5-25 ppm and 1-10 ppm of ZCE and its solvent fractions, respectively). These test solutions were separately formulated in 0.01% (*v*/*v*) ethanol and dispensed into respective beakers for the assays. A parallel experimental setup containing essential oil of neem rich in triterpenoid azadirachtin which is a potent larvicide was used as positive control while the negative control setup constituted mosquito larvae in water with 0.01% (*v*/*v*) ethanol. Larval mortalities in each experimental setup were recorded in each replicate for 24-h post-treatment. To assess the effects of ZCE and its active fraction (ZCFr.5) on larval development, 20 newly molted (synchronous) early L3 instars (4-5 days post egg hatching) added into three beakers were treated with sublethal dosages (ZCE: 8 ppm, 10 ppm; ZCFr.5: 1.0 ppm, 3 ppm for *An. gambiae*, and *Ae. aegypti*, respectively). Time taken for the mosquito larvae to reach pupal stages was recorded, and percentage pupation for each treatment replicate calculated (n = 3). Parallel setups constituting distilled water and 0.01% ethanol served as references.

3.12 Total RNA isolation, cDNA synthesis, and RT-*q*PCR analyses

Total RNA was isolated from pools of five mosquito larvae obtained from ZCFr.5-treated and non-treated control groups using TRIzol reagent (Invitrogen™, Carlsbad, CA 92008, USA) according to manufacturer's guidelines. To assess the purity and concentrations of the isolated RNA, a Nanodrop 2000 UV Vis spectrophotometer (Thermo Scientific, USA) was used. cDNA first strand was synthesized from DNase I treated total RNA (500 ng) using oligo $d(T)_{20}$ primers of SuperScript® IV First-Strand

Synthesis System (Invitrogen Life Technologies, Lithuania) as per manufacturer's instructions. Gene expression analyses were performed using Agilent Stratagene Mx3005P real-time *q*PCR system (Agilent Technologies, USA) with SYBR Green®/ROX *q*PCR master mix (Thermo Scientific, USA). All primers targeting the mosquito ecdysteriodegenic pathway CYP450 and JHAMT genes used for RT-*q*PCR analyses are listed in Appendix 7. Gene-specific amplification reaction comprised of 6.25 µL SYBR Green/ROX, 0.5 picomoles of forward and reverse primers, 1 µL of 1:5 diluted cDNA, and topped up to 12.5 µL with nuclease-free water. The PCR cycling conditions were programmed as follows; initial step 95°C for 10 min, 40 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec followed by a single cycle of dissociation melt curve set at 95°C for 30 sec, 55°C for 1 min and 95°C for 30 sec. Normalization of the gene expression levels was performed using species-specific ribosomal protein S7. All the experiments were performed three times. The relative gene expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

3.13 Biochemical acetylcholinesterase (AChE) enzymatic assay

Ellman's protocol (Ellman et al., 1961) with minor modifications was followed to measure the AChE inhibitory activities of ZCE. A pool of 10 larvae from each species was separately washed three times in phosphate buffer (1× PBS, pH 8.0) before homogenization in 1 mL PBS. The homogenates were centrifuged at 10,625 ×g for 20 min at 4°C and supernatants separately transferred into sterile clean 1.5-mL Eppendorf tubes to give the enzyme extract source for assaying. Into 150 µL of ice-cold PBS held in a 96-well plate, 10 µL of the enzyme extract was added before addition of 20 µL of the test extract preparations. The test extracts were dissolved in DMSO to a final concentration of 0.1% DMSO (*w/v*) in PBS. The reactants were incubated at room temperature for 10 min followed by the addition of 20 µL 0.4 µM acetylthioiodide (ATChI, BDH Chemicals Ltd, England) and 0.3 µM 5,5´-DTNB (Ellman's reagent, Sigma Aldrich, USA). This was followed by a 30-min incubation at room temperature and absorbance read out using an Eppendorf BioSpectrometer® Fluorescence at 412 nm. A set up comprising of enzyme extract, DTNB, and the buffer was used as blank while a

positive reaction constituted the enzyme extract, ATChI, and DTNB. A commercial chemical Propoxur (PESTANAL® analytical standard, Sigma Aldrich, USA) was included as the reference inhibitor. The assay was performed in four replicates and the % AChE inhibition expressed as a mean value of the replicates as given in equation (1) below;

$$\%\ Inhibition = (1 - \frac{Absorbance\ in\ sample\ reaction}{Absorbance\ in\ control\ reaction}) \times 100$$

3.14 Data analyses

Antimalarial data were analyzed by Graphpad Prism 7.00 (Prism for Windows, v. 7.00, GraphPad Software Inc., California, USA). A non-linear regression model for normalized relative fluorescent units (RFU) readouts was fitted against log10-transformed drug concentrations for sigmoidal dose-response plots to estimate IC_{50} values for asexuals. The parameters used were; four parameter logdose with a variable slope: Y=Bottom + (Top-Bottom)/(1+10^((LogIC_{50}-X)*HillSlope)) fitting. Gametocytemia levels for each compound concentration was expressed as percentage inhibition of DMSO-treated controls and plotted against log_{10}-transformed doses using nonlinear regression to calculate IC_{50} values as previously adopted (Wadi et al., 2018). To determine the effect of juliprosopine on female *Anopheles* mosquitoes' survival, Kaplan-Meier's survival analysis was performed. Mantel-Cox's Log-rank test was used to determine the differences between the survivorship of vehicle controls and treated females. A generalized non-linear regression model (*glm*) was fitted in R software (version 3.5.1) to estimate the half-maximal lethal (LC_{50}) and inhibitory (IC_{50}) concentrations for the mosquito larvae using probit link function and *dose.p* function in MASS package. Mean differences in experimental data were compared by one-way ANOVA with Tukey's post-hoc analyses or Student's t-test and *p* values of less than 0.05 considered statistically significant. Correlations between mean LC_{50}s and AChE inhibition was performed using Pearson's correlation coefficient (*r*). A *p* value of less than 5% was considered significant. All the generated graphical data presentations were organized in Adobe illustrator CS6.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Bioactive *Cissampelos pariera* (Menispermaceae) and *Prosopis juliflora* (Fabaceae) compounds targeting *Plasmodium* cytokinesis exhibit gametocytocidal activities

4.1.1 Introduction

Revitalized efforts in search of leads for development of next-generation transmission-blocking candidates to synergize malaria elimination appear to have apparently overlooked the pivotal role of natural products as bounteous source. A major contribution for this lag is the favourable introduction of the synthetic chemical libraries vis-à-vis natural products from which many researchers rely on for high-throughput discovery and subsequent hit optimization (Li & Vederas, 2009; Ginsburg & Deharo, 2011). This has led to reluctance and deprioritization to pursue natural resources during the 21st Century, in the boosting of the malaria therapeutic armamentarium (Wells, 2011; Cragg & Newman, 2013). This position has been supported by the finite number of plant-derived agents possessing either gametocytocidal or sporogonic inhibitory activities that includes mainly terpenoid derivatives (section 2.5.3). However, within this category, are the less-studied alkaloid scaffolds including the most promising natural antimalarials, such as quinine and its derivatives. More strikingly, only the DNA intercalative cryptolepine, an indoloquinoline alkaloid isolated from *C. sanguinolenta* (Forkuo et al., 2017), and indoloquinazoline tryptanthrins from *Isatis tinctoria* (Onambele et al., 2015) were reported to inhibit gametocytes.

In a bid to expand the existing chemical space for transmission-blocking drug interventions, this study explored a panel of selected 13 plants (Table 3.1), from which two organic extracts were discovered to promisingly inhibit *Plasmodium* asexual growth. This exciting finding is highly comparable to activities previously reported by Rukunga et al., (2009) at IC_{50} 5.85 µg/mL for *C. pariera* and 4.1 µg/mL for *P. juliflora* (Al-Musayeib *et al*., 2012). Roots of *C. pariera* are characterized to possess active

bisbenzylisoquinoline (BBIQ) and protoberberine alkaloids (Semwal et al., 2014) while piperidyl indolizidine alkaloids dominate in *P. juliflora*. A comprehensive investigation of antimalarial activity and the associated compounds for these two plants has been missing. The observed activity for these two plants was therefore extended to sexual stages, and for the first time, to best of knowledge, their compounds were studied for potential malaria transmission-blocking in this study. BBIQs alkaloids are associated with protein synthesis inhibition and DNA intercalation to inhibit kinetoplastids (Merschjohann et al., 2001). On the other hand, plant-derived indolizidines, including phenanthroindolizidines, are well characterized (Liu et al., 2011; Rahman et al., 2011; Du et al., 2019); possessing a wide array of effects such as antiparasitic, antimicrobial, plant growth regulatory, anti-inflammatory, anticancer among others.

The present study describes the discovery of *C. pariera* and *P. juliflora* as potential antimalarial sources, isolation and identification of the bioactives, activity profiling against NF54/field isolate gametocytes. Furthermore, stage-specificity of action against asexuals was performed providing insights into possible mechanisms of action. Inspired by bioinformatics approaches, target predictions led to identification of processes linking the observed treatment phenotypes. These findings provide foundations for further optimization of the identified leads into potent target-specific malaria transmission-blocking antimalarial agents.

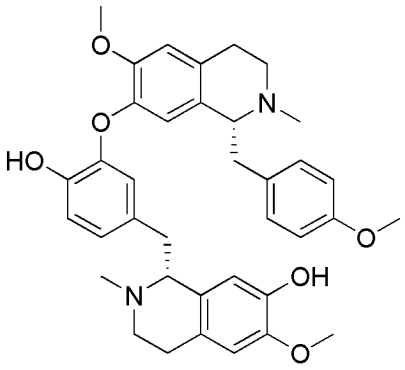
4.1.2 Results

4.1.2.1 Characterization of active antimalarial agents in *C. pariera* and *P. juliflora* extracts

Bioactivity-directed fractionation of active *C. pariera* and *P. juliflora* extracts using column chromatography over silica gel led to the isolation of highly active fractions CP08 and PJ10 within the aqueous methanol and water phases, respectively. With an exception of PJ10 that based on TLC profile suggested a discernible single compound as the major component juliprosopine (**87**), CP08 was re-chromatographed over silica gel into isolation of active antimalarial isoliensinine (**253**). The isolates were analyzed in the

positive mode of electron spray ionization mass spectrometry (ESIMS) in LC-MS/MS and the chemical data obtained used to compare and deconvolute the identity of the antimalarial compounds from published and online databases spectral information.

Isoliensinine (253)



Isoliensinine (**253**) was obtained as a yellowish brown amorphous powder with a molecular ion peak at *m/z* 611.2 $[M+H]^+$ at t_R 4.044 min and correspondence molecular formula $C_{37}H_{42}N_2O_6$ (calculated mass: 610.7326), indicating eighteen degrees of unsaturation. The compound from *C. pariera* displayed two prominent fragmentation ion peak characteristics at *m/z* 595.2 (44%), and 298.2 (70%) (Figure 4.1), associated with head-to-tail BBIQs (Baldas et al., 1972). Base peak (100%) had a fragmentation ion mass of 342.1. Through comparison with previously published spectral data (Bala et al., 2017), the compound was unambiguously identified as a BBIQ alkaloid, isoliensinine, previously isolated from *Nelumbo nucifera* and *Cissampelos*. Loss of NH_3 that led to the detection of fragment m/z 595.2 [M+H-16], and *m/*z 298.2 $[M+H-(237.1+16+43.9)]^+$ (CHO) designated a diagnostic fragmentation characteristic of isoliensinine.

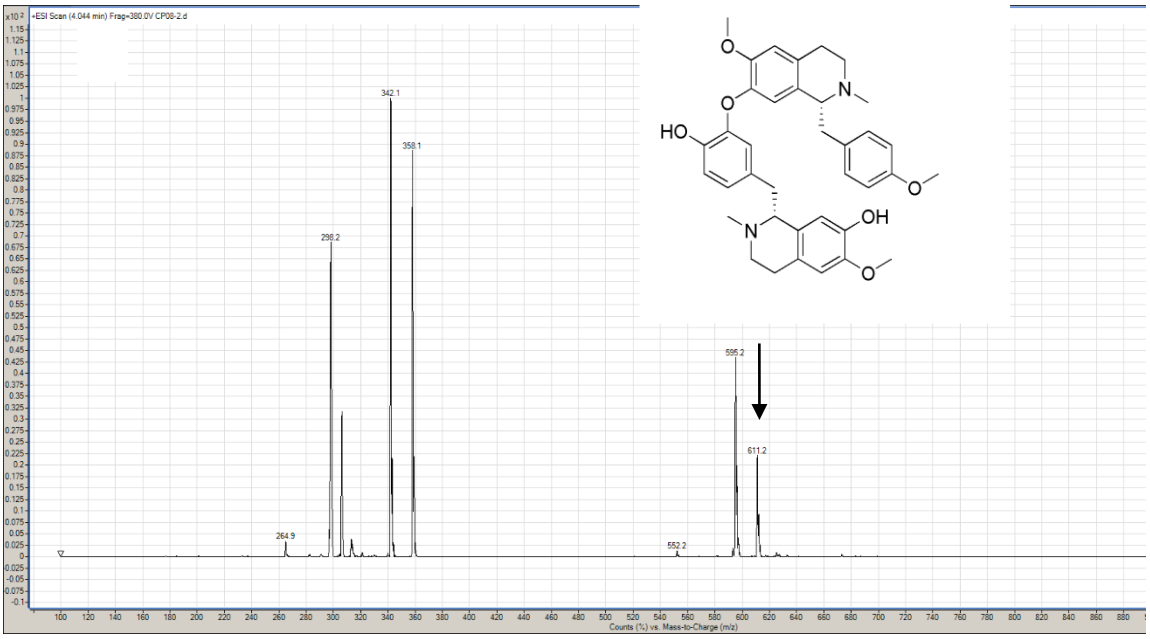
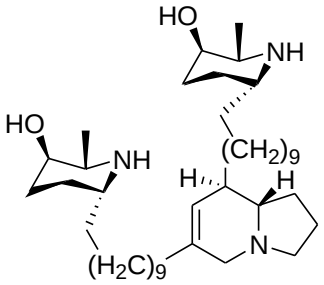


Figure 4.1: Full Scan product ion spectra of $[M+H]^+$ ions for Isoliensinine. Inset is the Isoliensinine chemical structure.

Juliprosopine (87)



Juliprosopine (**87**)

Compound **87** was obtained in a greenish yellow gum, soluble in water only. The parent ion ESI-MS/MS *m/z* 630.6 $[M+H]^+$ (calculated for $C_{40}H_{76}N_3O_2$; exact mass 630.5917) at t_R 6.476 min was assigned to a known indolizidine piperidyl alkaloid, juliprosopine, with four degrees of unsaturation. Two main molecular ESI-MS/MS fragment product ions at *m/z* 315.9 (100%) $[M+H-314.7]^+$ and, 211.0 (52%) $[M+H-(314.7+104.9)]^+$ were detected (Figure 4.2). This MS spectral data is in correspondence with that obtained by Singh &

Verma, (2012) and Batista et al., (2018). Consequently, the identified compound was deduced to be a 2,3-dihydro-*1H*-indolizinium heterocycle.

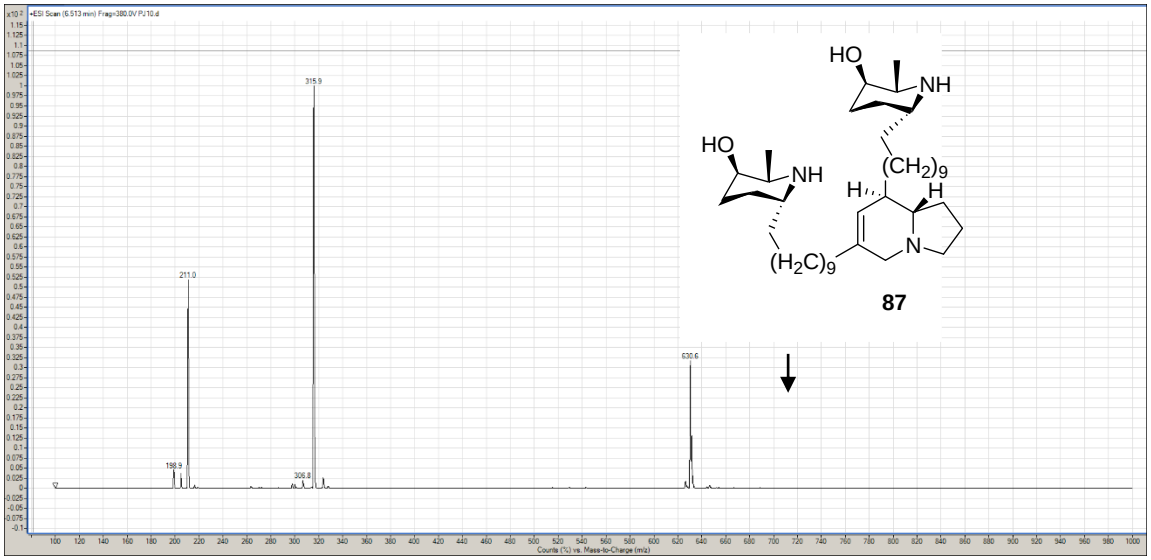


Figure 4.2: LC-ESI-MS/MS spectra fragmentation chromatogram of juliprosopine (87) from *P. juliflora* leaves. Inset is the chemical structure of juliprosopine.

4.1.2.2 Characterization of gametocytocidal effects of *C. pariera* and *P. juliflora* compounds

Following SYBR Green-based whole cell phenotypic screening of the 13 selected plant extracts against W2 strain, *C. pariera* and *P. juliflora* extracts were found to have mean IC_{50} values of 2.091 ± 0.1598 µg/mL and 1.017 ± 0.0245 µg/mL, respectively (Table 4.1).

Table 4.1 Antimalarial activity of plant extracts against asexual blood stages. SYBR Green I-based antimalarial screen readouts for relative fluorescence units (RFU) using Tecan GENious Plus (TECAN, Austria GmbH, Austria) plate reader at excitation and emission wavelengths of 485 nm and 535 nm, respectively. *Plasmodium* parasites (100 µL per well, 1% parasitemia and 2% hematocrit) were incubated for 72 h under standard culture conditions with test plant extracts serially diluted two-fold starting with final concentration of 50,000 ng/mL (12.5 µL dispensed per well). Parasite growth inhibitory concentrations at 50% (IC_{50}) were calculated by plotting relative fluorescent units (RFU) against logarithm concentrations in GraphPad prism 7.

Plant extract	***Plasmodium* W2 strain (IC_{50} µg/mL)**		**MDH 0038 (IC_{50} µg/mL)**
	Replicate 1	Replicate 2	Replicate 1
C. sinensis	> 1000	>1000	0.0089
M. koenigii	>1000	24.634	534.969
V. schiliebenii	432.771	>1000	0.4629
V. payos	24.651	6.338	901.937
P. Americana	174.69	>1000	24.361
M. indica	861.645	>1000	0.00496
A. conyzoides	19.386	52.632	0.063
P. juliflora	1.034	0.9993	0.0001264
S. terebinthifolius	106.098	52.764	8.621
T. brownii	42.926	14.840	9.408
C. cinerariifolium	603.363	17.211	4.260
Z. chalybeum	318.048	11.613	2.946
C. pariera	1.978	2.204	1.906

*Samples highlighted in yellow were chosen for further bioactivity characterization.

On subsequent activity-guided fractionations using a column chromatography, their solvent fractions, CP08 and PJ10, further displayed potent antimalarial activity against CQ-sensitive D6 (mean IC_{50} 1.329 ± 0.2775 µg/mL and 0.604 ± 0.2101 µg/mL, respectively). These solvent fractions further exhibited promising immediate *ex vivo* activity against clinical isolates (IC_{50} range 0.2-1.517 µg/mL; Table 4.2) albeit of slow speed-of-action, exerting inhibition between 48-72 h post-treatment (when tested against Dd2 strain; Figure 4.3). The activity was highly comparable to that exerted by primaquine (PQ, IC_{50} 0.3126 µg/mL) but less potent compared to dihydroartemisinin (DHA, IC_{50} 0.006 µg/mL).

Table 4.2 Antimalarial activity of *Cissampelos pariera* and *Prosopis juliflora* fractions against D6 asexual blood stages and three human clinical isolates. Three clinical *Plasmodium* isolates; KDH 0153, KOM 0145, and KOM 0148 were included for immediate *ex vivo* activity testing. These samples were collected from Kenya Ministry of Health registered Kisumu District Hospital and Kombewa sub-County Hospital, and processed within 4-6 h post-collection.

Fraction	***Plasmodium* D6 strain**	**KDH 0153**	**KOM 0145**	**KOM 0148**
	Mean IC_{50} µg/mL ± S.D	**IC_{50} µg/mL**	**IC_{50} µg/mL**	**IC_{50} µg/mL**
CP01	-	Inactive	72.722	Inactive
CP02	-	Inactive	Inactive	Inactive
CP03	-	Inactive	Inactive	18.451
CP04	-	Inactive	Inactive	24.709
CP05	-	Inactive	32.796	Inactive
CP06	-	31.405	Inactive	21.615
CP07	-	18.113	24.552	15.840
CP08	1.329 ± 0.2775*	1.517	1.078	0.2
PJ01	-	Inactive	Inactive	Inactive
PJ02	-	33.566	Inactive	Inactive
PJ03	-	Inactive	0.093	8.184
PJ04	-	Inactive	13.751	89.008
PJ05	-	15.46	Inactive	12.579
PJ06	-	Inactive	Inactive	Inactive
PJ07	-	Inactive	Inactive	Inactive
PJ08	-	Inactive	Inactive	Inactive
PJ09	-	Inactive	-	5.541
PJ10	0.604 ± 0.2101*	0.863	-	0.732
Primaquine	-	0.3126	-	-

The potent fractions from *Cissampelos pariera* and *Prosopis juliflora* and their activities are highlighted above in yellow color. – denotes not tested. * shows that the mean IC_{50}

values were statistically different (two-sample Welch t-test = 3.7843, df = 3.64, p = 0.02297).

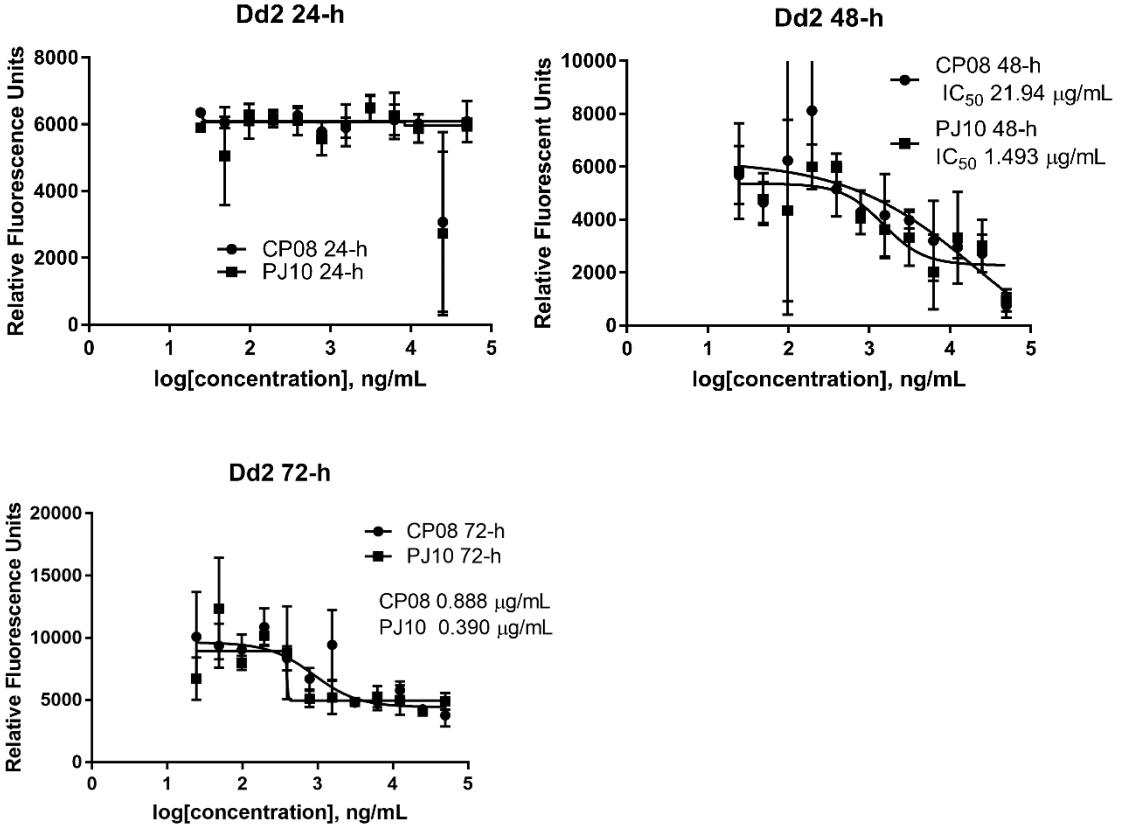


Figure 4.3. Time-specificity assay reveals slow speed-of-action of active fractions against *P. falciparum* Dd2 strain. Time course dose-response inhibitory kinetics of plant constituents action against Dd2 in four independent replicates of SYBR Green I IC_{50} speed assay (n = 4). Each point represents a mean value of the experimental replicates for the representative periods and error bars indicate standard deviation (s.d).

To identify the stage-specificity of *Plasmodium* growth inhibition, highly synchronized D6 parasites were treated at 12 h intervals of the 48-h intraerythrocytic cycle; rings (0-6 h post-invasion, hpi), trophozoites (18-24 hpi), and schizonts (36-42 hpi), and thin smears prepared at the end of each time point. It was observed that, relative to DMSO-treated parasites whose growth progressed perfectly from rings into multinucleated schizonts, transition from mature trophozoites into schizonts was arrested during the 1st

replication cycle on treatment with fractions CP08 and PJ10 constituents (Figure 4.4). This treatment phenotype is akin to that induced by inhibitors of plasmodial Serine/Arginine-rich protein-phosphorylating cyclin-dependent kinase-like kinases (*Pf*CLK 1-4) and coenzyme A biosynthesis (Fletcher & Avery, 2014; Kern et al., 2014; Alam et al., 2019). Having profiled the antimalarial activity of the two active plant extracts against the asexual stages, their potential to incapacitate various gametocyte stages by their constituents was examined using NF54/field isolate parasites.

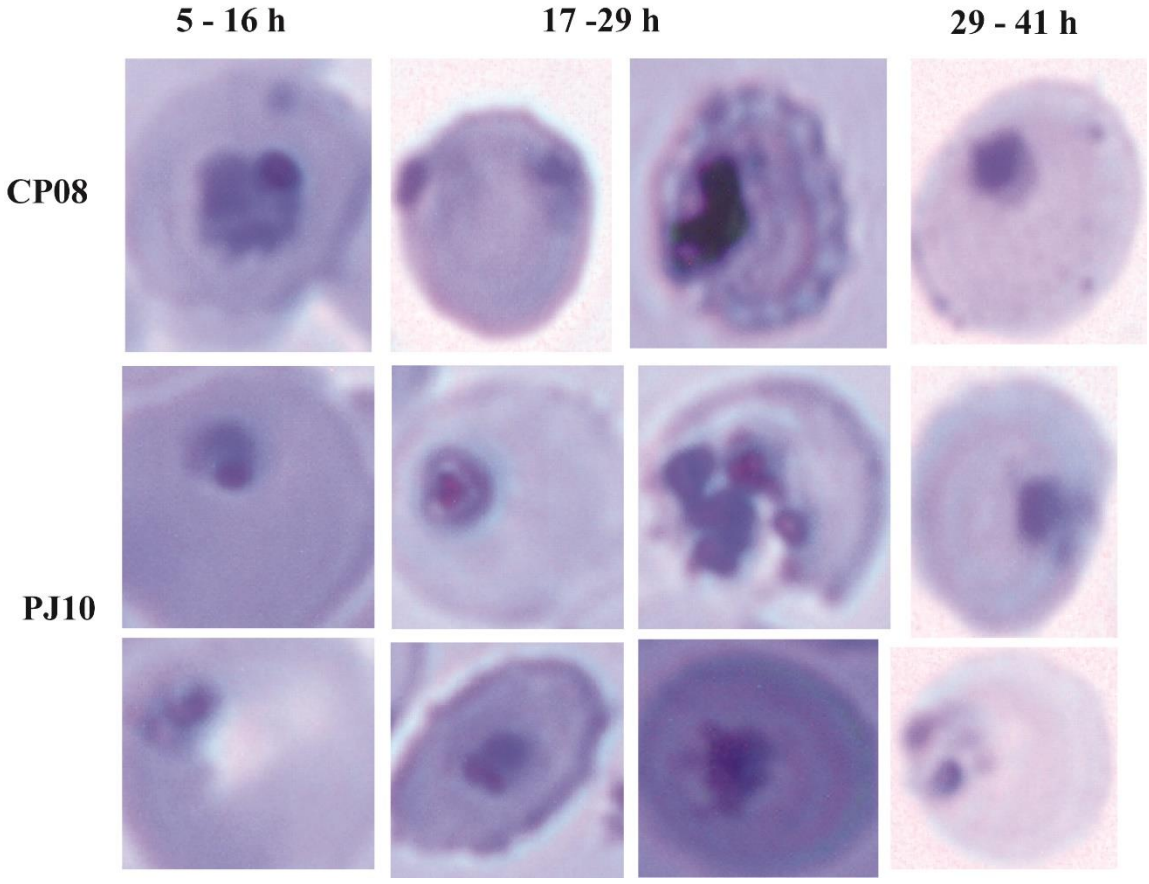


Figure 4.4. Stage specificity of action by *C. pariera* isoliensine and *P. juliflora* juliprosopine against *Plasmodium* asexuals. Representative images depicting stage specificity effects exerted against mature trophozoites and schizonts maturation. Smears were made after treatment with isoliensinine and juliprosopine. Aliquots of synchronized Dd2 parasites at 1% parasitemia ring stages were treated at 5-16, 17 – 29, and 29 – 41 hpi. Morphological examination by light microscopy, under 100× oil immersion

objective, was used to detect the stage specificity of action relative to DMSO-control treatment. Three independent experimental replicates were performed (n = 3).

The killing effect of juliprosopine and isoliensinine on mature late-stages IV/V gametocytes that are in readiness for maturation to infect mosquitoes was assessed by microscopy readouts. Incubations of the parasites for 72-h resulted into dose-dependent reduction in gametocytemia levels (Figure 4.5 A - D), with isoliensinine exhibiting a gametocytocidal IC_{50} 0.028-0.4319 µg/mL and nanomolar potency for juliprosopine IC_{50} 0.012 – 0.002 µg/mL. These findings translated into 3.7–fold potency selectivity against gametocytes over the asexuals. Unfortunately, PQ failed to give definite results as noted by Adjalley et al., (2011), and data was not shown. For comparisons, the recent *in vitro* IC_{50} value of PQ for stage V gametocytes reported to be 6.5 µM (Plouffe et al., 2016) was referenced. Focusing attention to juliprosopine, microscopic examination of gametocytes from juliprosopine-treated wells revealed intense morphological deformations (Figure 4.5E).

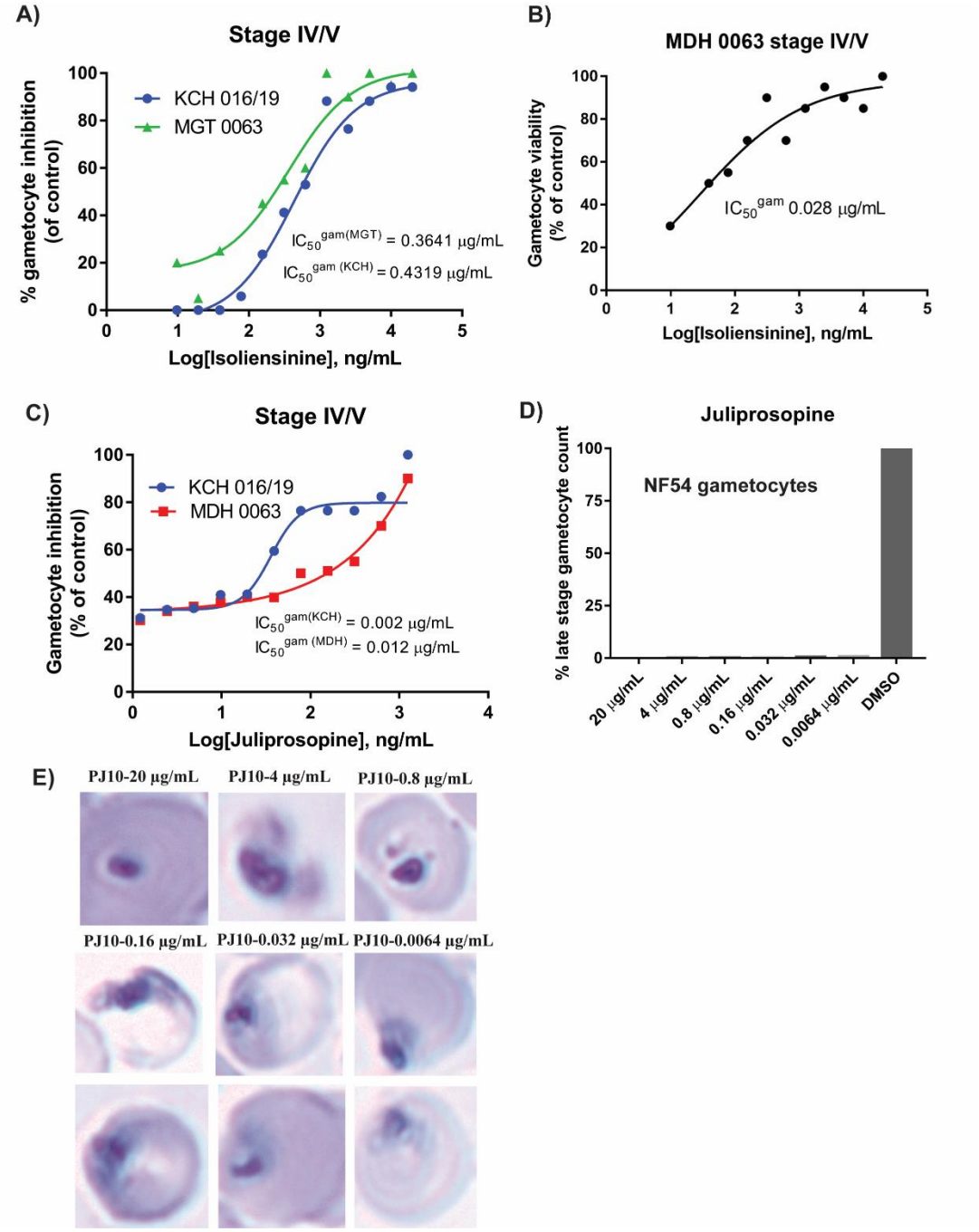


Figure 4.5: Gametocytocidal effects of *C. pariera*'s isoliensinine and *P. juliflora*'s juliprosopine. A-B: Dose-response curves for gametocytocidal activities of isoliensine (**253**) against field isolate late-stage IV/V gametocytes. **C-E:** Gametocytocidal activities of juliprosopine (**87**). The first figure C represents the dose-response curves for KCH 016/19 and MDH 0063 late-stage IV/V gametocytes. D: viable NF54 gametocytes count

following juliprosopine treatments. E: Morphological examination of gametocytes from various treatment dosages of juliprosopine.

Having established that *P. juliflora* juliprosopine potently kills the *P. falciparum* late-stage IV/V gametocytes, further evaluations on the extent of gametocytogenesis inhibitions were conducted. Treatment with either 5× IC_{50} (3.02 µg/mL) or 1× IC_{50} (0.604 µg/mL) of gametocyte cultures initiated with sexually-committed rings during daily medium feeds demonstrated remarkable gametocytogenesis blockade by day 7 post-induction (>95% clearance of stages I-III for 5× IC_{50} and > 80% for 1× IC_{50}). Interestingly, washout following addition of 5× IC_{50} for 72-h after gametocytogenesis onset >90% killed all the developing gametocytes by day 7 profiling as no viable parasites were observed (Figure 4.6 A & B), suggesting low recovery rates.

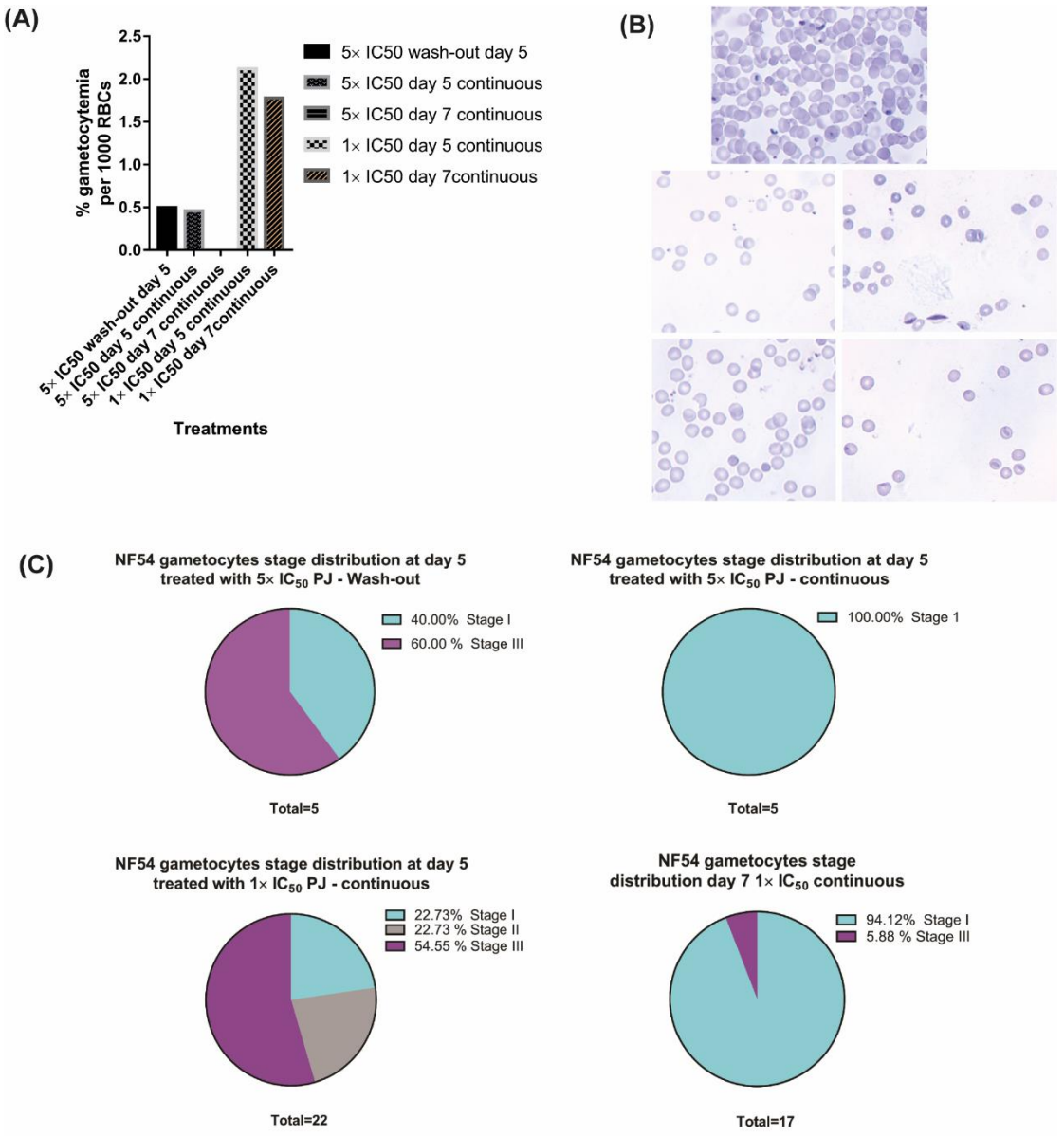


Figure 4.6: Gametocyte growth kinetics on supplementation with juliprosopine. *Plasmodium* parasites at 5-8% parasitemia levels were induced for gametocytogenesis in presence of potent juliprosopine at 1× IC_{50} and 5× IC_{50} concentrations. Spent media was replaced with freshly-dosed medium for 7 days, with smears prepared for gametocytemia assessment. **(A)** Gametocytemia levels at day 5 and 7. Data presented from three independent experimental replicates, $n = 3$. **(B)** Representative microscopic images of treated cultures at different time-points of gametocytogenesis. The topmost image represents the parasitemia level at the initial prior to gametocytogenesis induction (day 0). The second lane depicts gametocytes continuously treated with 1× IC_{50}

Juliprosopine and the third lane represents gametocytes either continuously treated at 5× IC_{50} or with wash-out after 2 days post-induction. (**C**) Relative distribution of early-stage gametocytes at day 5 and 7 profiling after treatment with juliprosopine.

4.1.2.3 Antimalarial activity of isoliensine and juliprosopine predicted to be associated with cell division regulatory proteins

BBIQs and indolizidine-bearing compounds are presumably believed to inhibit Ca^{2+}-dependent ATPases and mitotic activities (Tamburini et al., 1982). This is not precisely defined in protozoans. Information regarding their targets was gleaned computationally in this study. Using the publicly accessible databases, ChEMBL and PlasmoDB, Isoliensinine was highly predicted to interact with 55 eukaryotic target proteins (Appendix 1); with 21 (38.2%) lacking *Plasmodium* homologs. Of the 34 remaining target homologs, 27/34 (79.41%) (2 redundant proteins) are expressed during gametocyte development. These include: casein kinase 2 (Pf3D7_1108400), cysteine repeat modular protein 4 (Pf3D7_1475400), a putative metallo hydrolase/oxidoreductase protein (Pf3D7_0723700), calcium-dependent protein kinase 2 (Pf3D7_0610600), 3',5'-cyclic nucleotide phosphodiesterase beta (Pf3D7_1321500), RAC-beta serine/threonine protein kinase (Pf3D7_1246900), a putative mitochondrial chaperone BCS1 protein (Pf3D7_0603200), protein phosphatase PP2A regulatory subunit A (Pf3D7_1319700), bromodomain protein 2 (Pf3D7_1212900).

Other gametocyte proteins identified are sodium/hydrogen exchanger (Pf3D7_1303500), cytoadherence linked asexual protein 3.1 (Pf3D7_0302500), CDPK7 (Pf3D7_1123100), erythrocyte membrane protein 1 (Pf3D7_0712800), enoyl-acyl carrier reductase (Pf3D7_0615100), protein farnesyltransferase subunit alpha (Pf3D7_1242600), phosphatidylinositol 4-kinase (Pf3D7_0509800), a putative 3-oxo-5-alpha-steroid 4-dehydrogenase (Pf3D7_1135900), a putative aldehyde reductase protein (Pf3D7_1364600), V-type K^+-independent H^+-translocating inorganic pyrophosphatase (Pf3D7_1235200), erythrocyte membrane protein 1 (Pf3D7_1200600), calpain (Pf3D7_1362400), merozoite-associated tryptophan-rich antigen (Pf3D7_0830500),

bifunctional dihydrofolate reductase-thymidylate synthase (Pf3D7_0417200), dipeptidyl aminopeptidase 2 (Pf3D7_ 1247800), and a putative DnaJ protein (Pf3D7_ 1038800). Additionally, 12/34 (35.3%) regulate the *Plasmodium* cell-cycle; Pf3D7_1108400, Pf3D7_0518200, Pf3D7_1212900, Pf3D7_0809900, Pf3D7_0417200, Pf3D7_0610600, Pf3D7_1412100, Pf3D7_1362400, Pf3D7_1321500, Pf3D7_1319700, Pf3D7_1123100, and Pf3D7_1242600. At asexual stage, isoliensinine was established to inhibit trophozoite-schizont transition. Predicted targets at this stage of life-cycle were 17 (51.52%); Pf3D7_1108400, Pf3D7_1475400, Pf3D7_0723700, Pf3D7_1235200, Pf3D7_0610600, Pf3D7_0302500, Pf3D7_1123100, Pf3D7_0712800, Pf3D7_1038800, Pf3D7_1247800, Pf3D7_0417200, Pf3D7_0615100, Pf3D7_0830500, Pf3D7_1135900, Pf3D7_1362400, and Pf3D7_1200600. Functional classifications indicated that most of the predicted protein targets were involved in protein modifications (8), chromatin remodeling and cell-cycle regulation (6), RBCs invasion (5), fatty acid biosynthesis (3), and mRNA processing (2) (Figure 4.7A).

The target eukaryotic proteins associated with juliprosopine were 66; 23 (34.85%) lacked *Plasmodium* homologs (Appendix 2) and 2 had functional redundancy. Majority of these targets were functionally classified to be involved in protein modifications (9), chromatin remodeling and cell-cycle regulation (7), RBCs invasion (6), fatty acid biosynthesis (3), DNA synthesis (2), RNA metabolism (2), mitochondrial functions (2), and phosphoinositide metabolism (2) (Figure 4.7A), in that order. With an exception of PVP01_1331500, Pf3D7_1315100, PBANKA_1138400, Pf3D7_1445400, PF3D7_1008000, and Pf3D7_0920800, juliprosopine was predicted to share *Plasmodium* cell-cycle regulatory protein targets with isoliensinine. The predicted targets expressed during trophozoite-schizont were 23/41 (56.1%); Pf3D7_0100100, Pf3D7_ 1205700, Pf3D7_0712000, Pf3D7_0412400, Pf3D7_1434600, Pf3D7_1135900, Pf3D7_1128700, Pf3D7_1475400, Pf3D7_1431500, Pf3D7_1407900, PBANKA_1138400, Pf3D7_0610600, Pf3D7_0615100, Pf3D7_0417200, Pf3D7_0321500, Pf3D7_1140000, Pf3D7_1428800, Pf3D7_1212900, Pf3D7_0920800, Pf3D7_0400800, Pf3D7_0826100, PocGH01_09034900, and PFI0525w. With exception

of the 11 targets shared with isoliensinine (Figure 4.7B), juliprosopine was predicted to target other 21 gametocyte proteins; PBANKA_0909800, Pf3D7_0100100, Pf3D7_1205700, Pf3D7_0712000, Pf3D7_0412400, Pf3D7_1434600, Pf3D7_1128700, PVP01_0417700, PVP01_1412100, Pf3D7_1431500, Pf3D7_1407900, Pf3D7_0922900, Pf3D7_0321500, Pf3D7_1352100, Pf3D7_1140000, Pf3D7_1428800, Pf3D7_0920800, Pf3D7_0400800, Pf3D7_0826100, PocGH01_09034900, and PFI0525w.

Overall, besides showing differential antimalarial potencies, the two compounds shared 13 *Plasmodium* proteins; Bromodomain protein 2 (BDP2; Pf3D7_1212900), Calcium-dependent protein kinase 2 (CDPK2; Pf3D7_0610600), Phosphatidylinositol 4-kinase (PI4K; Pf3D7_0509800), Phosphodiesterase 4β (Pf3D7_1321500), Cysteine repeat modular protein 4 (Crmp4; Pf3D7_1475400), Bifunctional dihydrofolate reductase-thymidylate synthase (Dhfr; Pf3D7_0417200), 3-oxo-5-alpha-steroid 4-dehydrogenase, putative (Pf3D7_1135900), Calpain (PBANKA_1138400/Pf3D7_1362400), Pf3D7_0809900, Protein farnesyltransferase subunit alpha (Pf3D7_1242600), Rh5-interacting protein (Pf3D7_0323400), Aldehyde reductase, putative (Pf3D7_1364600), and Enoyl-acyl carrier reductase (ENR; Pf3D7_0615100), previously demonstrated as antimalarial drug targets (Figure 4.7A). However, considering the functionality of the predicted antimalarial targets, the two compounds apparently targeted similar parasite pathways (Figure 4.7A). These proteins were also shared for two compounds in the gametocytes (Figure 4.7B).

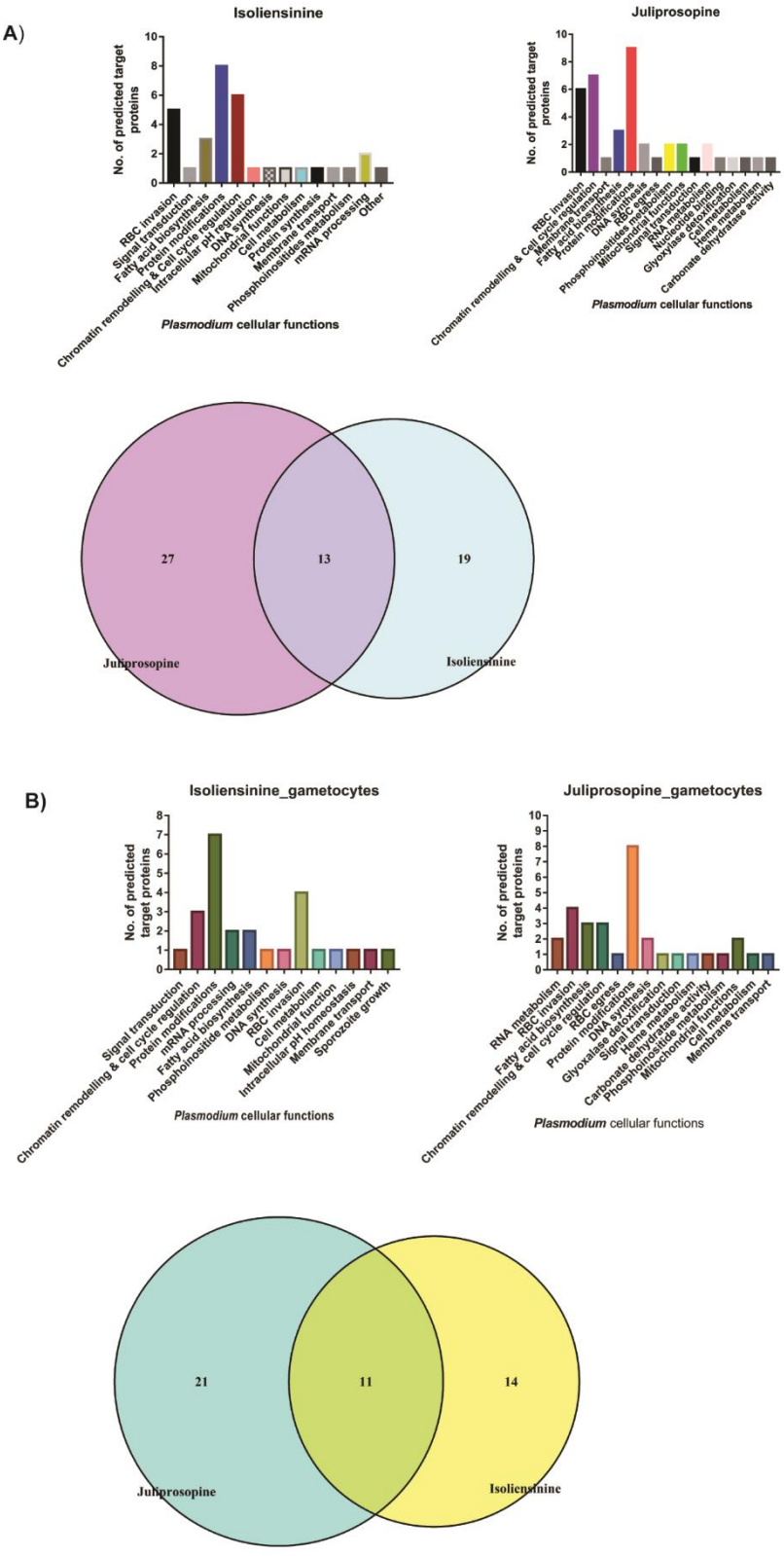


Figure 4.7: Predicted molecular targets of Isoliensinine (253) and Juliprosopine (87) in Plasmodium. Eukaryotic targets of the two antimalarial compounds were predicted in ChEMBL and protein sequences retrieved from publicly accessible and curated UniProt database (http://www.uniprot.org). The sequences were used to query PlasmoDB by *Blastp* for homologous proteins. The identified proteins were functionally classified into compound-specific for all the predicted targets, including shared targets by a venn diagram (A). Targets identified and expressed during gametocyte development for the two antimalarial compounds are presented (B).

Insights into ADME properties of the two antimalarial juliprosopine and isoliensinine were computationally predicted in SwissADME. Appendix 3 summarizes the correspondent physicochemical parameters for the two antimalarial agents. The predicted parameters indicated poor water solubility, high lipophilicity, and distinct toxicity alert for CYP450 isoforms. Moreover, the molecular weights were beyond the recommended Lipinski's limit of <500, requiring corrective strategies for drug-likeness.

4.1.2.4 Juliprosopine (87) exert little phenotypic effects on female *Anopheles* mosquitoes

Oral ingestion of sugar meals supplemented with potent antimalarial juliprosopine during the 15-day survivorship assay suggested little effects on female adults (Mantel-Cox Log-rank test; χ^2 = 0.541, df = 7, p = 0.9973), with treated and non-treated mosquitoes achieving equal survivorship levels irrespective of treatment dose (Figure 4.8A). These findings concur with the previous reports (Muller et al., 2017b; Sissoko et al., 2019) and that mosquitoes tolerate well with the antimalarial agent, indicating its tissue safety. When offered in bloodmeals at various concentrations, the mean egg numbers for the treated females at either 100 µg/mL (mean = 18 ± 1.4142) or 200 µg/mL (mean = 19 ± 1.4142) were not significantly different, but severely affected by 400 µg/mL (mean = 6 ± 2.1213) (Figure 4.8B). It was noted that some eggs from females ingesting bloodmeals containing 400 µg/mL juliprosopine lacked or had partial chorionic melanization, implying toxicity to oogenesis pathway. Relative to the controls that received blood with vehicle solvent, the overall female fecundity was greatly impaired irrespective of dosage. However, the mosquito larval hatching rates for the individual eggs remained comparable to the controls (Figure 4.8B).

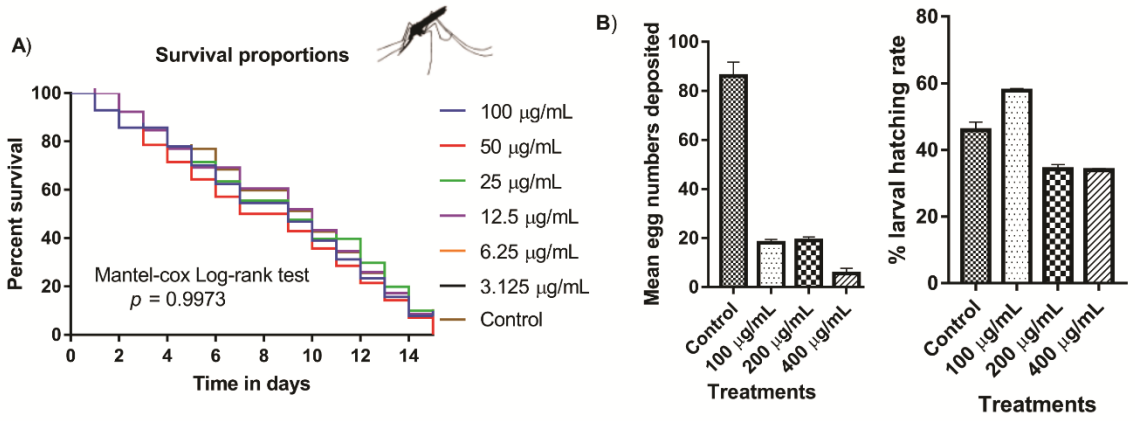


Figure 4.8: Effects of Juliprosopine on female *Anopheles* survivorship, fecundity and larval hatchability. A: Female adult mosquito survivorship following oral ingestions of juliprosopine formulations in sugar meal. Eighty (80) females/cage were assigned a treatment dose of between 3.125 µg/mL – 100 µg/mL or vehicle control offered via adsorbed cotton pads and daily mortality recorded for 15 days. Kaplan-Meier curve was generated from the mean values using Graphpad Prism 7. No significant differences in mean survival established between treatment and vehicle controls by Mantel-Cox Log-rank test (p = 0.9973). **B**: Effects of Juliprosopine on *Anopheles* fecundity and larval hatchability. Imbibed juliprosopine in bloodmeals severely impacted mosquito fecundity but not their hatchability. All the experiments were replicated twice and results expressed as mean values ± standard deviation.

4.1.3 Discussion

Insights into the inducible factors that smartly regulate *P. falciparum* gametocytogenesis and gametocyte maturation (Eksi et al., 2012; Kafsack et al., 2014; Sinha et al., 2014; Tibúrcio et al., 2015; Brancucci et al., 2017; Bunnik et al., 2018) are outpacing the discovery of potential inhibitors, a lag underscoring continued parasite transmissions. In fact, despite effectively inhibiting asexual replications, the existing antimalarials fail to suppress gametocyte production (Peatey et al., 2009; Adjalley et al., 2011). Only the spiroindolone NITD609 (van Pelt-Koops et al., 2012), plasmepsin V (WEHI-842) (Jennison et al., 2019), and CLK inhibitors (Kern et al., 2014; Alam et al., 2019) are described to arrest gametocytogenesis, in addition to demonstrating asexual growth inhibition and transmission-blocking activities. Attrition rates facing several HTS programs support prioritization of natural resource exploration for novel leads. Herein, the identification that plant-derived indolizidine and bisbenzylisoquinoline alkaloids potentially inhibit late-stage IV/V gametocytes and further schizont formation is described as a starting point for further development of novel malaria transmission-blocking arsenals.

The present study provides a detailed evaluation of isoliensinine (**253**) and juliprosopine (**87**) against *Plasmodium* parasites, albeit of initial antimalarial findings towards asexuals (Tshibangu et al., 2003; Rahman et al., 2011; Batista et al., 2018). An interesting additional feature of these compounds was however their strong selective submicromolar activity against the late-stage IV/V gametocytes, a less common attribute of the current antimalarials. The established dual-activity feature by these molecules perfectly matches the ideal outlined MMV portfolio of target candidate profiles (TCP-1 & 5) (Burrows et al., 2017). Being a BBIQ alkaloid resembling the antimalarial (-)-*O,O*-dimethylgrisabine from Malaysian *Dehaasia longipedicelata* (Lauraceae), it was not surprising to find isoliensinine (**253**) inhibiting asexual replications of multi-drug resistant backgrounds, inclusive of clinical human *Plasmodium* isolates. As previously reported (Marshall et al., 1994), antiparasitic BBIQs-based agents possess better activity in drug-resistant parasites by modulating CQ efflux pumps and synergistic action to

artemisinin. Consistent with these studies, isoliensinine (**253**) showed slightly better activity against multi-drug resistant Dd2 clone. However, to the best of our knowledge there is no existence of current data supporting isoliensinine (**253**), juliprosopine (**87**) and/or their structural analogs/bioisosteres as inhibitors of non-replicative *Plasmodium* forms. Therefore, the current findings provide the first evidence of their gametocytocidal activity alongside schizont formation inhibitions. While it may not currently be clear how the two compounds kill and/or inhibit gametocyte growth, the evidence that they dually inhibit asexuals and sexual stages signifies conserved targets across the two parasite stages. Indeed, compared to other previously reported plant-derived gametocytocidal compounds (subsection 2.5.3), isoliensinine (**253**) and juliprosopine (**87**) were highly comparable. Following the interesting finding that compound **87** achieved a selective nanomolar activity against late-stage IV/V gametocytes, morphological characterization of juliprosopine-treated gametocytes suggested selective parasite structural shrinkage, membrane distortions and nuclear condensations similar to those induced by methylene blue (Wadi et al., 2018). Gametocytes are susceptible to oxidant agents, for instance, artemisones and artemisides synergize methylene blue to impair redox homeostasis in *Plasmodium* (Coertzen et al., 2018). In that regard, juliprosopine (**87**) and isoliensinine (**253**) could herein act as metabolic poisons and permeant oxidants, killing the less metabolically active and impermeant late-stage IV/V gametocytes.

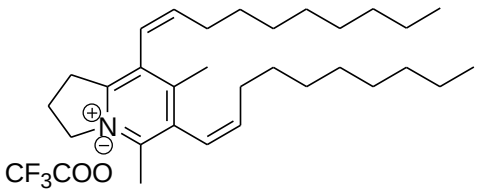
The most potent compound, juliprosopine (**87**), further blocked *Plasmodium* gametocytogenesis by day 7 profiling without severely affecting female mosquito fitness. Remarkably a similar outcome was observed on 3-day treatment following a wash-out condition. These reductions in gametocytemia were observed to be dose-dependent, with lower concentrations of the compounds permitting development of few gametocytes. Stochastic sexual conversions from a small number of gametocyte-committed asexuals generate infective gametocytes for mosquito transmissions, an epigenetically-regulated process involving ApiAP2 transcriptional switches (Kafsack et al., 2014; Sinha et al., 2014). To-date, antimalarial agents perturbing gametocytogenesis

are few. Identification of juliprosopine to exert gametocytogenesis blockade underscores the possibility of epigenetic network dysregulation, but also the involvement of other cellular mechanisms during gametocytogenesis cannot be disregarded. For instance, targeting protein processing and export by plasmepsin V proteases with a peptide mimetic WEHI-842 greatly blocked gametocytogenesis at stage II-V (Jennison et al., 2019). On the front of vector fitness impact, the compound **87** appeared less toxic as oral ingestion in sugar meals was well-tolerated. This finding agreeably matches the previous report that *P. juliflora* supplements mosquito sugar nutrition in the field (Muller et al., 2017). Whilst this reporting was directly derived from floral inflorescence data, the impact of the individual non-sugar indolizidine constituents has not been explored, but toxicity and/or counter nutritivity potential was herein unlikely. Remarkable fecundity impairment was recorded, with some eggs depicting incomplete melanization indicating probable impairment of phenylalanine metabolism and assimilation. In mosquitoes, disrupted phenylalanine metabolism through *AGAP005712* knockdown resulted in egg reduction and incomplete chorion melanizations by reducing available tyrosine for melanin synthesis (Fuchs et al., 2014). Such mechanisms that could underlie juliprosopine (**87**) action is however subject to further investigation by querying tyraminergic/alpha 2 adrenergic system and/or tyrosine bioavailability dynamics. The observation that similar larval hatchability rates were achieved relative to controls explains transient and discontinuous transfer of juliprosopine (**87**) treatment effects to embryonic development, although follow-up studies of offspring terminal effects are warranted.

How the molecules around these chemistries – indolizidines and BBIQs, impact protozoan parasites' biological processes affording killing effects, remain quite elusive to-date. Treatment with either isoliensinine (**253**) or juliprosopine (**87**) resulted in total inhibition of multinucleated schizont formation, perhaps by a common late-stage direct/indirect endomitotic cell division arrest mechanism. Microscopic examination of the treated pyknotic parasites at 29-h post-invasion revealed remarkable morphological changes including compact undivided nuclei, cell membrane distortions, bulged

cytoplasm, and disintegrated nuclear material. When added between 29-41 hpi, corresponding to schizont development duration, no multinucleated schizonts were observed that could trigger a new wave of RBC invasions. As a result, no noticeable impact was however recorded at parasite stages; early rings, late rings and early trophozoites. This activity profile greatly differs from that exerted by quinoline-based antimalarials including CQ, that target heme catabolism of all trophozoite stages. But, the treatment phenotype characteristics observed herein in addition to gametocyte killing effects are quite common with various experimental compounds targeting cytosolic protein synthesis (cladosporin, borrelidin; benzoxaboroles, AN6426 and AN8432)(Ishiyama et al., 2011; Hoepfner et al., 2012; Sonoiki et al., 2017), PI4K (imidazopyrazine, KDU691; MMV390048)(McNamara et al., 2013; Paquet et al., 2017), PfCLK 1-3 (oxo-β-carbolines, aminopyrimidines, and TCMDC-135051)(Kern et al., 2014; Alam et al., 2019), Na^+ ATPase 4 (Spiroindolone, KAE609)(Rottmann et al., 2010; Das et al., 2016), eEF2 (DDD107498)(Baragaña et al., 2015), Coenzyme A biosynthesis (Fletcher & Avery, 2014), Ca^{2+} oscillations (2-aminoethyl diphenylborinate, 2-APB)(Suárez-Cortés et al., 2017), folate metabolism (Pyrimethamine), and microtubules (Bell, 1998; Fennell et al., 2006). Indolizidines are building blocks of anticancer drugs vincristine and vinblastine, whose *Plasmodium* mitotic arrest activity has been previously reported (Usanga et al., 1986). These findings agreeably match the current study observations. Plausibly, Schneider et al., (2017) demonstrated that disruption of gametocyte microtubule network proteins (inner membrane complex (IMC)) impaired maturation, a possible target for compounds **87** and **253**. Contrastingly, Du et al., (2020) reported that indolizidinium alkaloid Anibamine (**295**; Figure 4.9) and its analogues from *Aniba citrifolia* (Lauraceae) barks recently exerted its antimalarial activity to rings and early trophozoite stages, likewise for BBIQ cepharanthine (Desgrouas et al., 2014). These remarkable differences in the stage specificity of action could be ascribed to compound motif variations whose targets might localize in different *Plasmodium* asexual stages. Recently, isoliensinine from *Nelumbo nucifera* was found to induce G1/S cell-cycle arrest in cancer cells by ROS generation

(Zhang et al., 2015). *Plasmodium* exhibits atypical mitotic division dissimilar from the canonical eukaryotic cell cycle (Francia & Striepen, 2014; White & Suvorova, 2018), and the cytokinesis arrest by compounds **253** and **87** could have occurred earlier (G1/S) and/or between S (DNA replication – late trophozoites) and M (asynchronous nuclear divisions at schizonts) phases. Furthermore, the disintegration of nuclear material, intracellular loss of osmotic regulation, and loss of cell membrane integrity translates into apoptotic inductions preceding parasite death. While these targets could postulate the possible mechanisms of action of compounds **87** and **253**, comprehensive delineation of the observed effects is highly warranted. As described by Sinden & Smalley (1979) using chemical inhibitors of cell cycle events, the late-stage IV/V gametocytes used in this study are usually at G_{2B} phase characterized by increased terminal repression of RNA and protein synthesis. This suggests that the gametocytocidal activities of juliprosopine (**87**) and isoliensinine (**253**) might be quite independent of DNA/RNA metabolism and protein synthesis, and based on other cellular modalities including cytoskeletal destabilization.



IC_{50} 0.170 µM against PfDd2

Anibamine **(295)**

Figure 4.9: Chemical structure of antimalarial Anibamine (295) from *Aniba citrifolia.*

In this study, a bioinformatic analysis in ChEMBL database predicted a number of compound-specific eukaryotic targets whose homologs functionally clustered overwhelmingly into well-known *Plasmodium* biological pathways; fatty acid biosynthesis, protein modifications, RBC invasions, and cell cycle regulations. Confirmatory target searches were cross-referenced to transcriptomic and proteomic data collected at late-trophozoites and stage IV/V gametocytes in PlasmoDB. The intricate

Plasmodium development is characterized by "just-in-time" stage-specific dramatic transcriptional, translational, and metabolic landscape re-organizations (Florens et al., 2002; Bozdech et al., 2003; Le Roch et al., 2004; Ke et al., 2015; Srivastava et al., 2016; Bunnik et al., 2018; van Biljon et al., 2019). Despite the transcriptional differences between asexual and sexual forms of *Plasmodium* parasites, it was noteworthy these cellular pathways appeared well represented in both parasite stages. That differentiation of late-trophozoites into multinucleated schizonts encompasses a major step to formation of infective merozoites and/or commitment into gametocytogenesis, key reported biological processes at this transition include; DNA synthesis, protein biosynthesis, proteosome degradations, apicoplast functions, and tricarboxylic acid cycle metabolic processes (Bozdech et al., 2003). Furthermore, late-stage IV/V gametocytes have been reported to notably express cytoskeleton, post-translational modifications, TCA cycle metabolism, lipid metabolism, mitochondrial redox, DNA replication, signal transductions, chromatin remodeling, cell cycle regulation, protein export and RBC surface remodeling, protein degradation processes (Khan et al., 2005; Tao et al., 2014; Lasonder et al., 2016; Miao et al., 2017). Underpinning the fact that the two antimalarial molecules exert cytokinesis arrest, their interaction with many regulatory protein processes encompassing reversible post-translational modifications (PTMs), could strongly underlie the observed treatment phenotypes. Active covalent post-translational protein modifications on *Plasmodium* chromatin and associated cytoskeletal proteins have been intensively implicated in guiding cytokinesis cell fates at schizont stage as well as controlling gene expressions in transmissible stages (Doerig et al., 2015; Yang & Arrizabalaga, 2017; Yakubu, Weiss, & de Monerri, 2018; Green et al., 2020). Owing to such an indispensable dependency of PTMs in *Plasmodium* cell division, virulence, and stage-specific transitions, efforts to identify small-molecule inhibitors targeting these cell modulatory pathways have shown great potential for antimalarial discovery (Baker et al., 2017; Alam et al., 2019; Coetzee et al., 2020). Predictive analysis further suggested interactions of compounds **87** and **253** with fatty acid biosynthetic proteins; 3-oxo-5-alpha-steroid 4-dehydrogenase (ECR, Pf3D7_1135900), 3-oxoacyl-[acyl-carrier-

protein] reductase (KAR, Pf3D7_0922900), and Enoyl-acyl carrier reductase (ENR, Pf3D7_0615100). Maintenance of parasite cytoskeletal structural reinforcement and generation of merozoites require precursor membrane assembly proteins. At sexual stages, lipids are highly implicated in gametocytogenesis for RBC surface remodeling, signal transductions, energy provisions, and sphingolipids membrane precursors (Gulati et al., 2015; Tran et al., 2016b). As such, disrupted fatty acid synthesis and elongation in the apicoplast translates into botched membrane biogenesis, structural defects, abrogated signal transductions, attenuated virulence, and eventual death.

A number of –omics datasets have reported the PfHP1-regulated ubiquitous expression of antigenic variation (*var*) and RBC invasion proteins throughout *Plasmodium* life cycle (Lopez-Rubio, Mancio-Silva, & Scherf, 2009; Brancucci et al., 2014). The predicted interactions with a number of host cell invasion and virulence proteins – PfEMP1, STEVOR, Pf3D7_0323400, Pf3D7_0302500, and RESA-like PHIST proteins could suggest reduction in host cell cytoadherence and remodeling associated with asexuals and late-stage IV/V gametocytes in RBCs (Tibúrcio et al., 2015; Dearnley et al., 2016; Neveu & Lavazec, 2019). As a critical feature associated with transmission (Aingaran et al., 2012), induced decreased RBC deformability coincides with low gametocyte survival and transmission potential, as demonstrated with sildenafil (Ramdani et al., 2015; Naissant et al., 2016). Such protein export to host cell targeted with plasmepsin inhibitors was recently demonstrated druggable inhibiting trophozoites and gametocytogenesis (Sleebs et al., 2014; Jennison et al., 2019). A number of proteins acting at cell cycle regulation and chromatin remodeling events were predicted to interact with compounds **87** and **253**; bromodomain protein 2, histone deacetylase 2, calpain, JmjC domain containing protein, and meiosis-specific nuclear structural protein 1. Abundant expressions of these histone modifying proteins have been observed during various life-cycle transitions, including mitotic division in schizonts, gametocytogenesis and gametogenesis (Miao et al., 2006), and their interaction outcomes are surprisingly not owing to the exerted activities. Similar effects were obtained on treatment of *Plasmodium* with histone deacetylase (HDAC) inhibitors, modulating chromatin

transcriptional gene regulations (Chaal et al., 2010; Trenholme et al., 2014). Such targets maintain *Plasmodium* heterochromatin and their interactions with the two compounds in addition to blockade of histone protein modifications could emphasize transcriptional deregulations, euchromatin inaccessibility, and chromosomal missegregation. While this study focused on the majorly represented biological processes possibly targeted by the two antimalarials, the involvement of the other identified predictions into the concerted actions is seemingly crucial.

Critical for further optimizations and development of lead candidates using iterative medicinal chemistry strategies based on these chemical models is the determination of their structural suitability in reference to cellular metabolic stability and pharmacokinetics. Predictive ADME profiles suggested that, while the molecules had attractive antimalarial activities, their drug-likeness was however unlikely. In that regard, corrective synthetic chemistry and molecular modelling strategies to improve their solubility, bioavailability, and reduce the molecular weights are extremely recommended. Nevertheless, taken together based on the study findings, the two antimalarial scaffolds provide further exploration chemical starting points for development of novel transmission-blocking agents.

4.2 Neurotoxic *Zanthoxylum chalybeum* root constituents invoke mosquito larval growth retardation through ecdysteroidogenic CYP450s transcriptional perturbations

4.2.1 Introduction

Renewed interests in search of environmentally friendly alternative insecticides have lately led to the gradual substitution of chemical-based insecticides in global markets. However, as much as the public demand for biocides over their synthetic counterparts continues to increase and appreciation of plant-derived bioactive compounds in pest control grows considerably (George et al., 2014; Lengai, Muthomi, & Mbega, 2019; Muema et al., 2017), the mechanistic effects mediated by these insecticidal agents at a molecular level and their target proteins remain largely unresolved. Only studies focusing on phytophagous insects highlight structurally-based concerted cellular interference of vital physiological processes by growth-reducing plant compounds (Mithöfer & Boland, 2012). Conversely, there are few detailed molecular toxicity studies to elucidate the mechanisms of action of various mosquitocidal agents. In a recent study, tea proanthocyanidins interfered with mosquito larval growth and reproduction fitness by physiologically disrupting the juvenile hormone biosynthetic pathway (Muema et al., 2017). Using a yeast two-hybrid reporter system, Lee et al., (2015) showed that juvenile hormone mimics derived from *Lindera erythrocarpa* (Lauraceae) and *Solidago serotina* (Asteraceae) effectively antagonized mosquito juvenile hormone receptor, methoprene-tolerant (Met); killing mosquito larvae and retarding follicle development in the ovaries. Larvicidal activity of annonaceous acetogenin (squamocin) was associated with multitarget midgut gene effects in *Aedes aegypti* larvae (da Silva Costa et al., 2016). In other studies, terpenes and terpenoids, polyphenols, alkaloids, and phenylpropanoid compounds were found to target the larval neurotransmission process, inducing sudden neuronal toxicity and death (Hematpoor et al., 2016).

The post-embryonic insect molting, developmental timing, and morphological remodelling events controlled by periodical ecdysteroid pulses are under neural regulatory and nutritional inputs (Koyama & Mirth, 2018; Niwa & Niwa, 2014). These

metamorphosis behavioral changes are orchestrated by biosynthesis and release of growth hormones upon decoding sensory environmental cues from insulin/insulin-like signaling and target of the rapamycin (IIS/TOR) pathway. Ecdysteroids synthesized through sequential enzymatic oxidation of dietary cholesterol in the prothoracic glands are released into the hemolymph where they are activated into 20-hydroxyecdysone (20E) (Ou et al., 2016). Major advancements have been made to understand ecdysone functions in insect physiology. For instance, through comprehensive molecular analyses in model insects (e.g. *Drosophila melanogaster*) and other related species, it is evident that the ecdysteroidogenic pathway is transcriptionally regulated by Halloween genes that encode CYP450 enzymes; *Neverland*, *non-molting glossy* (*nmg*), CYP307A1/*spook*, CYP307A2/*spookier*, CYP306A1/*phantom*, CYP302A1/*disembodied*, CYP315A1/*shadow* and CYP314A1/*shade*, and a number of nuclear transcription factors (Niwa & Niwa, 2014, 2016; Pankotai et al., 2010). Following ecdysone activation to 20E within the peripheral tissues by *shade*, it binds to the ecdysone receptor (EcR) forming a heterodimeric complex with Ultraspiracle (USP). The resultant trimeric complex 20E/EcR/USP binds ecdysone response elements (EcREs) activating transcriptional expression of 20E-inducible genes; *E74*, *E75A*, *HR3*, *Broad*, *βFtz-F1*, and other downstream proteins involved in metamorphosis and morphogenesis (Fletcher & Thummel, 1995). Biosynthesis and release of ecdysteroids from neurosecretory cells in metamorphosing juvenile insects could apparently be halted under a modulatory chemical environment, underscoring the anti-ecdysteroid-inducing effects of certain xenobiotic stressors.

In multicellular organisms, the neuronal coordination network of nerve circuits is regulated by a serine hydrolase acetylcholinesterase (AChE; E.C 3.1.1.7), which rapidly terminates synaptic signals by hydrolyzing the neurotransmitter, acetylcholine (ACh), into inactive derivatives (Toutant, 1989). Besides this primary function, the implication of AChE in atypical noncholinergic roles (Soreq & Seidman, 2001) including the regulation of insect growth and development is characterized. Exemplified by this important functional role in cellular development and survival, AChE continues to be

explored in biotechnological and chemical-based control of crop pests and insect vectors (Pang et al., 2012; Dou et al., 2013; Mutunga et al., 2019). As a biochemical target of organophosphates and carbamates, through phosphorylation or carbamoylation of a conserved serine residue (Ser200), AChE delineates a classical pest control chokepoint, despite its vulnerability to point mutations associated with decreased insecticide sensitivity. Various RNA interference (RNA*i*) studies targeting *Ace* of diverse insects including *Helicoverpa armigera* (Noctuidae), *Chilo suppresalis* (Crambidae), *Plutella xylostella* (Plutellidae), *Tribolium castenum* (Tenebrionidae), *Bemisia tabaci* (Aleyrodidae), fall armyworm *Spodoptera frugiperda* (Noctuidae), and *Bombyx mori* (Bombycidae) have reported adverse effects on larval growth and survival, delayed pupation and adult emergence, reduced motor control and female reproductive viability (Kumar, Gupta, & Rajam, 2009; He, Sun, & Li, 2012; Hui et al., 2011; Lu et al., 2012; Malik et al., 2016; Saini et al., 2018; Ye et al., 2017), implicating a significant role of AChE in insect growth physiology. Most likely, the mentioned findings could postulate a direct involvement of AChE in the regulation of insect hormonal activities. In fact, previous studies in metamorphosing brain of *Tenebrio molitor*, and cell lines of *Chironomus tentans*, *Ae. aegypti*, *S. frugiperda*, and *D. melanogaster* have demonstrated a relationship between AChE activity and its regulation by ecdysteroid hormone levels and vice versa (Cohen, 1981; Spindler-Barth et al., 1988; Jean-Jacques, Jean-Paul, & Jean, 2005; Jenson, Paulson, & Bloomquist, 2012). Studies using *D. melanogaster* and *Manduca sexta* as models have further provided significant insights into how insect larval-pupal transition behavior through ecdysis and tissue differentiation is driven by motoneuron networks controlled by ecdysteroid hormone and EcR activity (Novicki & Weeks, 1993; Truman, 1996; Veverytsa & Allan, 2013). Based on these interactions and interdependent co-regulatory mechanisms during growth, transcriptional dysregulation of either AChE or ecdysteroid genes by chemical intervention, RNA*i* and/or genetic ablation could adversely affect hormone-regulated insect growth by inducing toxicity and retardation phenotypes of inter-instar and larval-pupal transitions. In spite of this

knowledge, the underlying molecular mechanisms responsible for the growth retardation effects exerted by inhibitory influences on insect AChE remain unclear.

Underscoring the public health importance of mosquitoes in the transmission of life-threatening diseases, particularly malaria and arboviral infections, vector control aiming at area-wide suppression of adult populations are reconsidering targeting the juvenile larval stages (Tusting et al., 2013). This is largely due to the ever-growing concerns of insecticide resistance and other consequential behavioral effects associated with intensified adult vector interventions. Due to cost and environmental concerns associated with synthetic mosquito larvicides, community-based vector control interventions have shown great interest in the application of naturally occurring botanicals for mosquito control around human dwellings (Demissew et al., 2016; Gianotti et al., 2008; Imbahale & Mukabana, 2015; Trudel & Bomblies, 2011). In that regard, intensified laboratory screening of plant derivatives have reported a number of effective larvicides, among them derived from *Zanthoxylum* plant species (Kim & Ahn, 2017; Moussavi et al., 2015; Overgaard et al., 2014; Pavela & Govindarajan, 2017; Zhang et al., 2009). While several plant-derived AChE antagonists are potent insecticides, the knowledge of how they functionally affect insect larval development is underexplored. There are no existing studies of *Z. chalybeum* (knobwood) bioactivity against mosquitoes and therefore the current study reports the effects of its root chemical constituents on developing *An. gambiae* and *Ae. aegypti* juveniles. We demonstrate that dysregulation of mosquito larval nervous coordination upon exposure to ZCE root extract and its bioactive fraction (ZCFr.5) retards larval-pupal transitions through transcriptional perturbation of ecdysteroidogenic CYP450 regulatory genes and effector transcription factors.

4.2.2 Results

4.2.2.1 ZCE and its bioactive fraction are neurotoxic and retards mosquito larval development in a dose-dependent manner

Previous studies have demonstrated inhibition of *An. gambiae*, *Ae. aegypti*, *Culex pipiens*, and *Ae. albopictus* mosquito larval development by *Zanthoxylum* plant extracts (

Zhang et al., 2009; Overgaard et al., 2014; Moussavi et al., 2015; Kim & Ahn, 2017; Pavela & Govindarajan, 2017). Currently, mosquito larvae exposed transiently to ZCE at 15-25 ppm were immobilized and less active within the first 20 min for both *An. gambiae* and *Ae. aegypti*. This exposure elicited 60-100% larval mortalities at LC_{50} 9.00 ppm (95% CI 8.62-21.50) and LC_{50} 12.26 ppm (95% CI 9.04-19.80) against *An. gambiae* and *Ae. aegypti*, respectively, within 24-h post-treatment (Table 4.3). When compared to the negative controls that achieved 100% survival, ZCE significantly reduced larval survival rates (*An. gambiae*, $F_{(5,24)} = 105.5$, $p < 0.001$; *Ae. aegypti*, $F_{(5,24)} = 314.4$, $p < 0.001$) but slightly higher doses were required to match the activity of neem oil that was included as a positive control. However, the variation in susceptibility to ZCE toxicity was insignificant ($F_{(1,48)} = 0.836$, $p = 0.365$). Fractionation of ZCE yielded a bioactive hexane-soluble fraction (ZCFr.5) with similar activity to ZCE but at lower doses of LC_{50} 1.58 ppm (95% CI 0.62-2.87) and 3.21 ppm (95% CI 2.99-3.83) against *An. gambiae* and *Ae. aegypti*, respectively (Table 4.3). Other solvent fractions yielded no activity (Appendix 4).

Table 4.3: Toxicity of ZCE extracts on *An. gambiae* and *Ae. aegypti* larvae 24-h post-exposure.

Treatments	**Concentration (ppm)**	**Larval mortality (% mean ± S.D)**		**LC_{50} (95% CI)**	
		An. gambiae	*Ae. aegypti*	*An. gambiae*	*Ae. aegypti*
MeOH extract (ZCE)	25	100 ± 0.00[a]	100 ± 0.00[a]	9.00 (8.62-21.502)	12.26 (9.04-19.799)
	20	94 ± 1.64‡	79 ± 2.280[a]		
	15	78 ± 3.36‡	62 ± 1.140[a]		
	10	66 ± 1.82‡	37 ± 1.643[a]		
	5	38 ± 3.36[a]	2 ± 0.894[a]		
Hexane soluble fraction (ZCFr.5)	10	100 ± 0.00[a]	100 ± 0.00‡	1.58 (0.62-2.87)	3.21(2.99-3.83)
	7.5	94 ± 2.28[a]	87 ± 0.447‡		
	5	90 ± 1.12[a]	75 ± 2.387[a]		
	2.5	78 ± 0.98[a]	24 ± 3.162[a]		

	1	25 ± 2.23[a]	10 ± 1.673[a]
Neem oil (positive control)	3.8	98 ± 1.59	100 ± 0.00

Late third instar mosquito larvae of *An. gambiae* and *Ae. aegypti* were exposed to various doses of ZCE (5-25 ppm) and its active fraction (1-10 ppm) and mortalities in each setup recorded for 24-h against the controls. Mosquito population size (N) per treatment dose per replicate was 125 larvae (n = 2 biological replicates). Mean values followed by a double dagger (‡) are not significantly different. Values followed by a superscript letter are significantly different ($p < 0.05$, One-way ANOVA – Tukey-Kramer's multiple comparison test). ppm denotes parts per million, S.D – standard deviation, CI – confidence interval, LC_{50} – lethal concentration on 50% of test population.

Additionally, the residual effect of long-term exposure on developmental growth progression was evaluated. Upon treatment with sublethal dosage of either the ZCE or hexane-soluble bioactive fraction (ZCFr.5), the larval transition to pupal stages was abnormally extended (averagely 3.75 ± 0.1732 days, Table 4.4) inducing retardant phenotypes (ZCE: *An. gambiae* t = 15.963, p = 0.0004243; *Ae. aegypti* t = 23.883, p = 0.0004457; ZCFr.5: *An. gambiae* t = 30.311, p = 0.001087, *Ae. aegypti* t = 7.0162, p = 0.01272). Further, unlike the controls that achieved > 82% pupation (Table 4.4), pupation rate for the treated cohorts was significantly affected with many dying at larval state (ZCE: *An. gambiae* t = -18.826, p = 0.0007721; *Ae. aegypti* t = -19.078, $p < 0.0001$; ZCFr.5: *An. gambiae* t = -14.233, p = 0.0001549, *Ae. aegypti* t = -31.787, p = 0.0006135). Overall, species-specific perturbations were noted, with more treatment effects in *An. gambiae* than *Ae. aegypti* (ZCE, t = -11.539, p = 0.0005719; ZCFr.5, t = -6.3535, p = 0.02296).

Table 4.4: Developmental duration of mosquito larvae treated with sublethal doses of *Z. chalybeum* extracts.

Treatment	Pupation time (days)‡	Pupation rate (%)
An. gambiae		
Non-treated control	5.30 ± 0.17^{a}	85.33 ± 3.10^{a}
0.01% ethanol control	5.60 ± 0.20^{a}	82.61 ± 3.89^{a}
ZCE	9.10 ± 0.00^{b}	37.22 ± 1.51^{b}
ZCFr.5	9.40 ± 0.36^{b}	34.73 ± 1.72^{b}
Ae. aegypti		
Non-treated control	4.80 ± 0.35^{c}	89.40 ± 2.71^{c}
0.01% ethanol control	5.00 ± 0.26^{c}	86.15 ± 1.90^{c}
ZCE	8.90 ± 0.10^{d}	54.65 ± 2.14^{d}
ZCFr.5	8.80 ± 0.90^{d}	50.55 ± 0.39^{d}

‡Time period recorded from early L3 instar until pupal transformation for three replicates. Values presented as means ± standard deviation (S.D). Mean values followed by similar superscript letters are not significantly different (One-way ANOVA – Tukey-Kramer's multiple comparison test, $p < 0.05$). Sublethal dosages used: ZCE: 8 ppm, 10 ppm; ZCFr.5: 1.0 ppm, 3 ppm for *An. gambiae*, and *Ae. aegypti*, respectively.

4.2.2.2 Phytochemical analysis identified an abundance of insect growth reducing compounds

To identify the chemical constituents present in the bioactive hexane-soluble fraction (ZCFr.5), the yellow-brown oily sample was subjected to a gas chromatography-mass spectrometer (GC-MS) analyzer. From a total of 67 components (Appendix 5), the GC-MS analyzer detected among other compounds the presence and abundance of previously reported insect growth-reducing agents (Zhang et al., 2016; Kim & Ahn, 2017), corresponding to 2-tridecanone (**296)** (Rt 18.2267), hexadecanoic acid (**297)** (Rt 23.3864), (*Z,Z*)-9,12-octadecadienoic acid (linoleic acid) (**298)** (Rt 25.0829), sesamin (1,3-Benzodioxole,5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-,[1S-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-) (**299)** (Rt 34.3786) and β-caryophyllene (**300)** (Rt 38.38)(Figure 4.10A, 4.10C). Proportionately, the compounds **296**, **297**, **298**, **299**, and **300** contributed to 10.82%, 7.71%, 11.14%, 11.43%, and 10.38%, respectively, representing 51.48% of the total active biomass (Figure 4.10B).

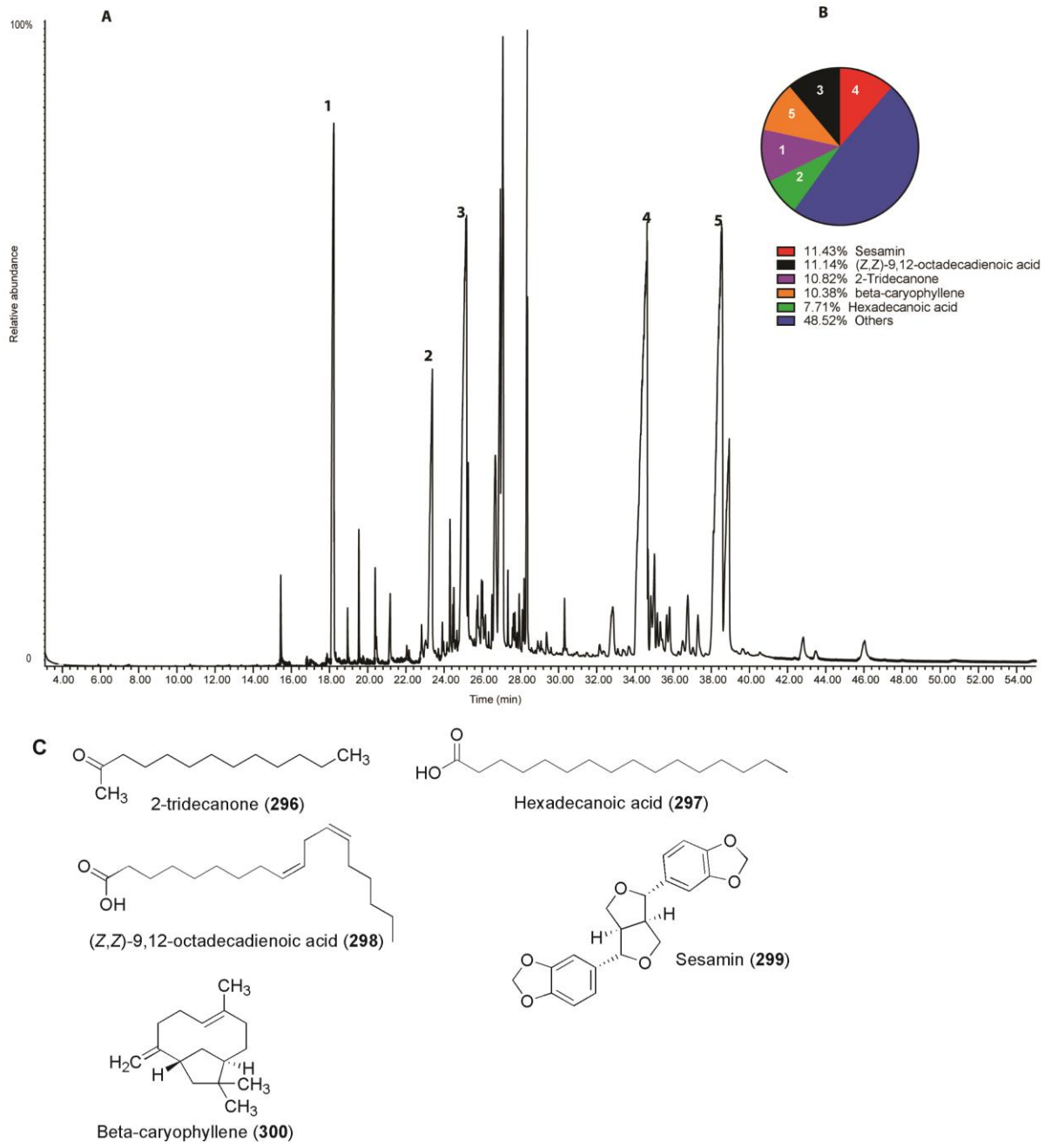


Figure 4.10: Analysis of chemical constituents in bioactive fraction. (A): The total ion chromatographic of GC-MS analysis spectrum from hexane-soluble *Z. chalybeum* root fraction. (**B)**: Relative proportions of the major compounds in bioactive biomass. (**C)**: Chemical structures of the identified major compounds, (**296**): 2-tridecanone, (**297**):

hexadecanoic acid, (**298**): (*Z,Z*)-9,12-octadecadienoic acid, (**299**): sesamin, and (**300**): β-caryophyllene.

4.2.2.3 Perturbed acetylcholinesterase (AChE) activity linked to larval lethality

Transient exposure of mosquito larvae to ZCE and its bioactive fraction for <5 h exhibited no obvious morphological aberrations but drastically reduced larval swimming behavior, induced muscle paralysis and immobilization leading to death. These observations are closely linked to neuromuscular toxicity effects reported by Tomé et al.(2014). To ascertain whether the observed acute toxicity on mosquito larvae was as a result of suppressive effects of ZCE and its bioactive fraction on AChE, the biochemical colorimetric Ellman's enzymatic assay and RT-*q*PCR gene expression analysis were performed. Due to the low amount of ZCFr.5, only ZCE was assayed for larval AChE activity inhibition. ZCE exhibited dose-dependent AChE inhibitory effect in mosquito larvae (Figure 4.11B), attaining IC_{50} 136.0 µg/mL (95% CI 111.3-547.3) and 277.5 µg/mL (95% CI 47.4-293.4) for *Ae. aegypti* and *An. gambiae*, respectively. The AChE inhibition achieved by ZCE was 1.4-2.73 fold higher in *Ae. aegypti* (44.19-81%) than *An. gambiae* (16.11-59.59%). Irrespective of ZCE dosage, the toxicity susceptibility of AChE significantly varied between *Ae. aegypti* and *An. gambiae* (Welch two-sample t-test, $p < 0.001$), highlighting a species-specific response. Relative to propoxur, a specific carbamate-based irreversible AChE inhibitor, the mean ZCE activity varied significantly between the species (*An. gambiae*; t-test, t= -8.4174, df = 4, $p = 0.001055$; *Ae. aegypti*; t=-2.9963, df = 4, $p = 0.03648$). Agreeably, the larval treatment with ZCFr.5 was associated with 0.0764 and 0.017-fold *Ace* transcriptional changes in *Ae. aegypti* and *An. gambiae*, respectively (Figure 4.11A), but these expressions were not significantly different from each other (t-test = 2.4406, df = 4, $p = 0.066$). Meaningful and significant ($p > 0.05$) negative correlation coefficients between the mean LC_{50} and AChE inhibition values of ZCE were obtained, but not varied between the two mosquito species (Appendix 6).

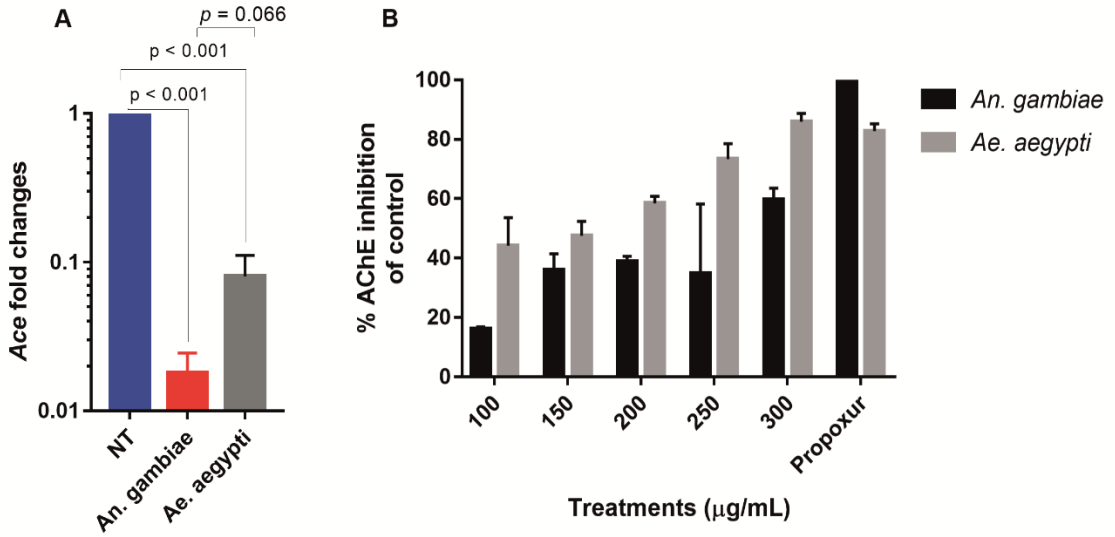


Figure 4.11: Effects of larval treatment on acetylcholinesterase activity. (**A**): Change of AChE gene (*Ace*) expression following larval treatment with ZCE bioactive fraction (ZCFr.5) within 25 min. Variation in expression compared by Student's t-test for three independent replicates (n = 3). NT denotes non-treated control whose gene expression was adjusted to 1 for both mosquito species. (**B)**: Normalized ZCE inhibitory activity of mosquito larval AChE (data presented as % mean ± S.D of n = 2 biological replicates, 4 technical replicates). Propoxur was tested at 100 µg/mL.

4.2.2.4 ZCFr.5 modulates mosquito larval ecdysteroid biosynthetic and transcriptional regulatory genes

Lignan-based phenylpropanoid and 2-tridecanone compounds are believed to inhibit ecdysteroids and their biosynthetic-dependent cytochrome P450 activities (Harmatha & Dinan, 2003; Zhang et al., 2016), interfering with insect growth. As expected, the sesamin-rich ZCE root fraction altered the transcriptional expression of ecdysteroid biosynthetic Halloween and regulatory genes (Figure 4.12). Notably, the larvicide treatment significantly down-regulated the expressions of larval 'black box' cytochrome P450 *spook* (*Cyp307a1*), *βFtz-F1*, *disembodied* (*Cyp302a1*), and ecdysteroid receptor (*EcR*) genes. It was further noted that, with the exception of *Cyp302a1* in *Ae. aegypti*, the gene expressions of *Cyp314a1*, *Cyp306a1*, and *dHAT* were not significantly different from each other (t-test, $p > 0.05$; Figure 4.12A-B), but remarkably modulated relative to the controls. Intriguingly, while juvenile hormone (JH) expression levels decrease during

the last larval instar to allow 20E-induced transformation into pupal stages, this study established that the expression of JH biosynthetic rate-limiting enzyme, JH acid *O*-methyltransferase (*JHAMT*), in ZCFr.5-treated larvae remained relatively higher compared to that of controls (Figure 4.12C-D). This finding could suggest low levels of circulating hemolymph 20E to suppress JH and further underscoring the observed larval growth retardations and precocious pupations. These findings confirmed that indeed the larval treatment that targeted AChE activity also perturbed ecdysteroidogenic pathway associated genes delaying larval-pupal transitions and inducing retardant phenotypes.

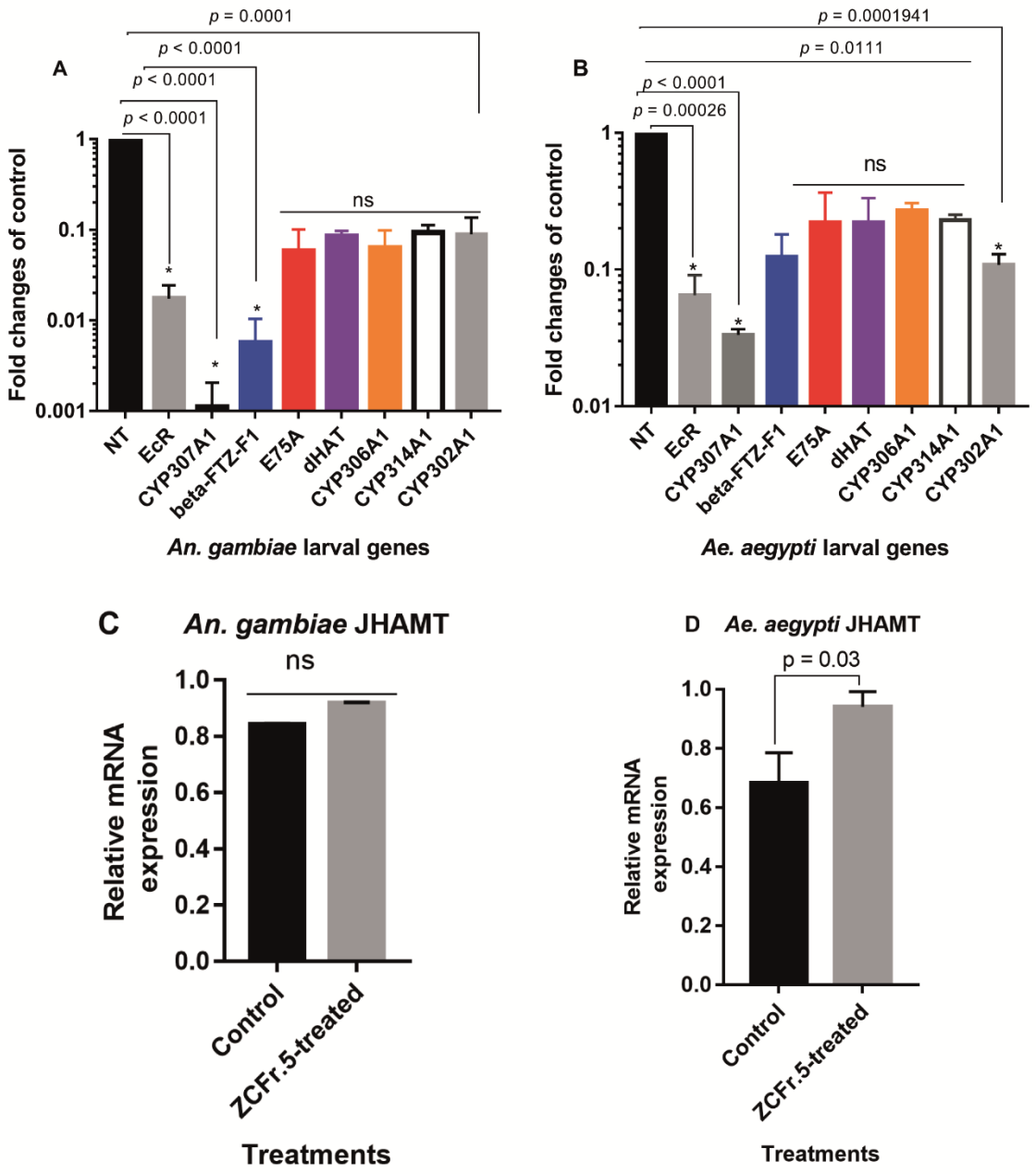


Figure 4.12: Ecdysteroidogenic transcriptional gene profiles on larval treatment. A -B: Mean fold changes in gene expression levels of ZCFr.5-treated *An. gambiae* and *Ae. aegypti* larvae. Mosquito larvae were treated at sublethal doses (slightly below species-specific LC_{50} 1 ppm for *An. gambiae* and 3 ppm for *Ae. aegypti*) for 72-h and gene expression assessed using RT-*q*PCR. Experimental controls were incubated with a solution containing 0.01% ethanol (*v/v*) in distilled water. Significant alterations in larval

gene expressions were noted (*t-test*, $p < 0.01$). *indicates statistically significant data at $p < 0.05$, *ns* - not significant from each other (Student's t-test), NT – Non-treated control whose gene expression was adjusted to 1 for both mosquito species. All the experimental assays were performed in triplicates ($n = 3$). **C - D**: Expression profiles of *JHAMT* in ZCFr.5-treated larvae relative to non-treated control groups. Data are expressed as mean ± S.D of experimental replicates.

4.2.3 Discussion

The impact of plant-derived insecticidal compounds on insect tissues elicits pleiotropic growth effects for which a number of insecticides specifically targeting neuromuscular activity have been overlooked at the molecular level. Herein the bioactivities of *Z. chalybeum* root constituents against *An. gambiae* and *Ae. aegypti* larvae were investigated with a keen focus on elucidating the possible downstream toxicity effects. The findings demonstrate that the fast-acting ZCE constituents elicit larvicidal activities in a dose-dependent fashion by targeting the neuromuscular actions regulated by AChE and further invoking larval retardation at sublethal doses through disrupted ecdysteroidogenesis. While similar observations have previously been reported using RNA*i* assays in other insects (He et al., 2012; Hui et al., 2011; Kumar et al., 2009; Lu et al., 2012; Malik et al., 2016; Saini et al., 2018; Ye et al., 2017), the study reports for the first time the effect of ZCE root constituents to dysregulate neural-linked ecdysteroidogenesis in mosquito larvae, inducing retardants that fail or exhibit delayed pupation.

Zanthoxylum plant species have been reported to possess insecticidal, repellent and larvicidal activities exerted by compounds mostly concentrated within the hexane-soluble portions (Kokate, Venkatachalam, & Hassarajani, 2001; Kamsuk et al., 2007; Tiwary et al., 2007; Matasyoh et al., 2011; Talontsi et al., 2011; Overgaard et al., 2014; Moussavi et al., 2015; Liu et al., 2014; Wang et al., 2015; Kim & Ahn, 2017; He, Wang, & Zhu, 2018). Similarly, *Z. chalybeum* larvicidal activity reported in this study was more pronounced in hexane-soluble fraction suggesting that the lipophilicity parameter was key in mediating the observed acute toxicity effects. Compared to their reported bioactivities exerted at relatively higher LC_{50} doses, ZCE and its active fraction (ZCFr.5) provoked larval toxicities at low LC_{50} doses (1.58-12.26 ppm) against *An. gambiae* and *Ae. aegypti*, possibly due to species-specific distribution of the secondary metabolites. While different compounds from the stem barks, seeds, and leaves were reported to differentially interfere with mosquito survival, this study detected for the first time the presence and abundance of sesamin, 2-tridecanone, (*Z,Z*)-9,12-octadecadienoic acid

(linoleic acid), hexadecanoic acid (palmitic acid), and β-caryophyllene in *Z. chalybeum* root extract that have previously been reported to exhibit growth reducing effects in insects. For instance, the phenylpropanoid lignan, sesamin, has been reported in *Zanthoxylum* stem barks to elicit moderate larvicidal activity (LC_{50} >150 µg/mL)(Moussavi et al., 2015) but relatively with similar bioactivity to ZCE against *Ae. aegypti* at 14.28 mg/L (Kim & Ahn, 2017). Further, an aliphatic methyl ketone (2-tridecanone) exhibited repellent effects against both *Amblyomma americanum* (Ixodidae) and *Dermacentor variabilis* (Ixodidae) ticks, and *An. gambiae* (Innocent et al., 2011). Strikingly, this carbonyl compound (2-tridecanone) retards insect growth by down-regulating Halloween CYP450 genes (Harmatha & Dinan, 2003), a feature corroborated in the current study. In addition, the ubiquitous caryophyllene and fatty acids, such as linoleic acid and palmitic acid, have previously exhibited larval toxicity mediated through AChE inhibition (Perumalsamy et al., 2015). Ingestion and/or cuticular penetration of this blend of compounds presumably interacted with larval tissues, thus incapacitating mosquito larvae during 24-h of treatment. Considering the larvicidal activity of ZCE compounds appeared to be associated with muscle paralysis manifesting in uncoordinated motility, inhibition against AChE appeared to be as a result of the lipophilic nature of these compounds that transverse cell membranes to their target site, possibly increasing ACh levels and overstimulating neuromuscular action akin organophosphates. These findings agree with those of Calderón et al. (2001), that reported acute toxicity of Mexican *Gutierrezia microcephala* (Asteraceae) on *S. frugiperda* larvae through AChE inhibition, in addition to reduced growth and delayed pupation.

Residual growth effects are often associated with insecticides but have largely been underexplored for *Zanthoxylum* extracts and other insecticidal compounds with AChE inhibition properties. Lack of any induced morphological aberrations associated with insect growth regulators at sublethal concentrations motivated our hypothesis that the ZCE/ZCFr.5 treatment could have profound effects on the ecdysteroid pathway inducing larval retardation. The post-embryonic insect metamorphosis, associated with various

morphological and behavioral changes particularly during the late third instar of larval-pupal transitions, is accompanied by ~100-fold neurogenesis and neural remodeling events for the maturation of adult-specific neuronal lineages (Kent et al., 1995; Truman & Reiss, 1995). It has been demonstrated using various insect models that this phenomenon is coincidentally linked to ecdysteroid hormone activity regulated by stage-specific expression of biosynthetic Halloween CYP450 genes (Li & Cooper, 2001; White & Ewer, 2014). Since decreased mRNA levels of neuronal AChE gene could positively correlate with the observed changes in 20E biosynthetic enzymes and the fact that ecdysteroid receptor isoforms (EcR-A and EcR-B1) have been previously found in insect neuronal cells (Schubiger et al., 1998; Truman et al., 1994), toxicity perturbation of these neurosecretory cells by ZCE constituents could have resulted in reduced biosynthesis, release and signaling, insufficient to influence ecdysone-mediated larval growth and pupation. These findings, akin to nicotinic acetylcholine receptor (nAChR) neonicotinoid effects, depicted that reduced expression of AChE influenced larval-pupal transition by the perturbing ecdysteroidogenesis pathway. It is possible to postulate that tissue injuries on the developing larvae imparted by the larvicide treatment could have severely constrained ecdysteroid biosynthesis, hemolymph titres and signaling, arresting growth to allow regeneration as previously observed in *Drosophila* (Hackney et al., 2012). Furthermore, it could be possible that these cholinergic injuries had negative effects on larval prothoracic gland (PG) innervations among them the 5-hydroxytryptamine (5HT)-producing serotonergic neurons (Shimada-Niwa & Niwa, 2014) modulating inositol 1,4,5-triphosphate/calcium ($InsP_3/Ca^{2+}$) signaling pathway and reducing downstream ecdysone production that deprived pupal transformation. Nevertheless, the perturbed expression of steroidogenic CYP450s and especially *spook* (*Cyp307a1*) in the hypothetical rate-limiting "black box" clearly suggested either a partial or an absolute inhibition of 7-dehydrocholesterol (7dC) conversion to 5β-ketodiol resulting into diminished activation of *EcR* and effector signaling transcription factors *E75A* and *βFtz-F1*. In fact the genetic loss and/or RNA*i*-mediated gene silencing of *spook* results in slowed development and molting interference that impairs growth

(Namiki et al., 2005; Sugahara et al., 2017) due to low ecdysteroid titres as the prothoracicotropic hormone (PTTH) target SPOOK translation and phosphorylation in the "black box" for ecdysteroidogenesis. Additionally, the low hemolymph 20E titers in treated larvae were accompanied by slightly high JH levels (Figure 4.12C-D) that agreeably sustained the phenotypic expression of larval retardants. Disruption of enzymes and genes associated with the ecdysteroid biosynthetic and signaling pathway by plant-derived compounds has been investigated extensively (Dinan, 2001). Therefore, treatment with lignans and neolignans disrupts insect development by destabilizing steroid hormones (Harmatha & Dinan, 2003). Sesamin inhibits CYP3A4 by antagonizing the steroid nuclear receptor (Lim et al., 2012), and its presence and abundance in ZCE could have had significant inhibitory effects on ecdysteroid CYP450s and 20E response effectors due to its methylene dioxy phenyl (piperonyl) structure. Moreover, the previously observed retardant effects caused by plant-derived 2-tridecanone on *H. armigera* as a result of down-regulation of 20E biosynthetic genes, particularly *Cyp307a1* could also be involved in this study (Harmatha & Dinan, 2003). Previous studies further demonstrated inhibitory regulation of CYP450s by saturated and unsaturated fatty acids (Hirunpanich et al., 2007; Palacharla et al., 2017), and the exogenous presence of linoleic acid and palmitic acid alongside other compounds in the hexane-soluble fraction could interfere with hormonal metabolism in developing mosquito larvae.

These findings demonstrate that *Z. chalybeum* root constituents are acutely toxic to mosquito larvae. This toxicity being mediated through disruption of neuromuscular coordination and further dysregulating ecdysteroidogenic-associated CYP450s at sublethal doses deserves further toxicological investigations aimed at designing potential mosquito control agents. Additionally, with the promising larvicidal effects herein demonstrated, the findings propose a comprehensive environmental safety assessment of ZCE constituents on non-target organisms prior to the commercial formulation, recommendation, and application in disease vector control programmes.

CHAPTER FIVE

GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The current study aimed at finding new potential malaria transmission-blocking agents, either acting against the *Plasmodium* parasite and/or the mosquito vectors. Thirteen (13) selected plants from various parts of Kenya were considered for evaluations, leading to; (i) the identification of two potent antimalarial extracts from *Prosopis juliflora* and *Cissampelos pareira* rhizomes possessing gametocytocidal effects against *P. falciparum* parasites.

(ii) Fractionation of the organic extracts resulted into a bisbenzylisoquinoline (BBIQ) isoliensinine (**253**) from *C. pariera* rhizomes and indolizidine alkaloid juliprosopine (**87**) from *P. juliflora* leaves that mediated gametocytocidal activity. The two antimalarial compounds were further active on late *Plasmodium* trophozoites blocking transition into multinucleated schizonts, and further killing late-stage IV/V gametocytes. Many cell cycle regulatory proteins facilitate schizont formation, predicted computationally to interact with isoliensinine (**253**), and juliprosopine (**87**). Majority of these predicted targets clustered in important *Plasmodium* biological processes; protein modifications, cell cycle and chromatin remodeling, fatty acid biosynthesis, and RBC invasion, all functioning during late trophozoites and late-stage IV/V gametocytes. The most potent indolizidine alkaloid juliprosopine (**87**) further blocked *Plasmodium* gametocytogenesis (~99% inhibition) at day 7 post-induction profiling, suggesting a potential dual-stage active antimalarial lead despite two violations of Lipinski's rules (Mw > 500; MlogP > 4.15).

(iii) Further investigations demonstrated tissue safety and selectivity of juliprosopine in female mosquitoes, only impacting negatively on fecundity.

(iv) *Zanthoxylum chalybeum* root constituents that comprised major compounds; 2-tridecanone (**296**), hexadecanoic acid (**297**), (*Z*,*Z*)-9,12-octadecadienoic acid (**298**), sesamin (**299**), and β-caryophyllene (**300**) elicited fast mosquito larval lethality mediated by neurotoxicity. Sublethal dose treatments of mosquito larvae with the bioactive

fraction extended larval-larval inter-instar and larval-pupal transitions by modulating ecdysteroidogenic CYP450s genes.

5.2 Recommendations

In view of the findings of the current study, the following recommendations are made;

(1) Further exploration of isoliensinine (**253**) for antimalarial activity against *Plasmodium* gametocytogenesis and its interaction with juliprosopine (**87**). This includes the validation of the gametocytocidal activity using standard membrane feeding assays as well as functional validation of the predicted molecular targets in trophozoites and gametocytes.

(2) Optimization of the potent juliprosopine (**87**) and isoliensinine (**253**) by iterative medicinal chemistry strategies for structural-activity-relationships (SAR) elucidation and generation of candidate antimalarial leads

(3) Further interrogation of the molecular mechanisms underlying the impact of juliprosopine (**87**) on mosquito fecundity and follow-up offspring attributes for generational effects

(4) Determining the contribution of the major *Z. chalybeum* root constituents on mosquito larval lethality in substractive bioassays and successive hit-to-lead developments.

REFERENCES

Abay, S. M., Lucantoni, L., Dahiya, N., Dori, G., Dembo, E. G., Esposito, F., Lupidi, G., Ogboi, S., Ouédraogo, R. K., & Sinisi, A. (2015). *Plasmodium* transmission blocking activities of *Vernonia amygdalina* extracts and isolated compounds. *Malaria Journal*, *14*(1), 288.

Abraham, M., Massebo, F., & Lindtjørn, B. (2017). High entomological inoculation rate of malaria vectors in area of high coverage of interventions in southwest Ethiopia : Implication for residual malaria transmission. *Parasite Epidemiology and Control*, *2*(2), 61–69.

Adebayo, J. O., & Krettli, A. U. (2011). Potential antimalarials from Nigerian plants: A review. *Journal of Ethnopharmacology*, *133*(2), 289–302.

Adia, M. M., Emami, S. N., Byamukama, R., Faye, I., & Borg-Karlson, A.-K. (2016). Antiplasmodial activity and phytochemical analysis of extracts from selected Ugandan medicinal plants. *Journal of Ethnopharmacology*, *186*, 14–19.

Adjalley, S. H., Johnston, G. L., Li, T., Eastman, R. T., Ekland, E. H., Eappen, A. G., Richman, A., Sim, B. K. L., Lee, M. C. S., & Hoffman, S. L. (2011). Quantitative assessment of *Plasmodium falciparum* sexual development reveals potent transmission-blocking activity by methylene blue. *Proceedings of the National Academy of Sciences*, *108*(47), E1214–E1223.

Aguiar, A. C. C., Figueiredo, F. J. B., Neuenfeldt, P. D., Katsuragawa, T. H., Drawanz, B. B., Cunico, W., Sinnis, P., Zavala, F., & Krettli, A. U. (2017). Primaquine - thiazolidinones block malaria transmission and development of the liver exoerythrocytic forms. *Malaria Journal*, *16(1)*, 110.

Aguiar, A. C. C., Mm, E., Souza, N. B. De, França, T. C. C., & Krettli, A. U. (2012). New approaches in antimalarial drug discovery and development - A Review. *Memorias Do Instituto Oswaldo Cruz*. *107*, 831–845.

Aguilar, R., Magallon-Tejada, A., Achtman, A. H., Moraleda, C., Joice, R., Cisteró, P., Li Wai Suen, C. S. N., Nhabomba, A., Macete, E., Mueller, I., Marti, M., Alonso, P. L., Menéndez, C., Schofield, L., & Mayor, A. (2014). Molecular evidence for the

localization of *Plasmodium falciparum* immature gametocytes in bone marrow. *Blood*, *123*(7), 959–966.

Ahmad, A., Khan, K. A., Ahmad, V. U., & Oazi, S. (1986). Antibacterial activity of juliflorine isolated from *Prosopis juliflora*. *Medica (Mex.)*, *1*, 339.

Ahmad, V. U., Sultana, A., & Qazi, S. (1989). Alkaloids from the leaves of *Prosopis juliflora*. *Journal of Natural Products*, *52*(3), 497–501.

Aingaran, M., Zhang, R., Law, S. K., Peng, Z., Undisz, A., Meyer, E., Diez-Silva, M., Burke, T. A., Spielmann, T., Lim, C. T., Suresh, S., Dao, M., & Marti, M. (2012). Host cell deformability is linked to transmission in the human malaria parasite *Plasmodium falciparum*. *Cellular Microbiology*, *14*(7), 983–993.

Akala, H. M., Eyase, F. L., Cheruiyot, A. C., Omondi, A. A., Ogutu, B. R., Waters, N. C., Johnson, J. D., Polhemus, M. E., Schnabel, D. C., & Walsh, D. S. (2011). Antimalarial drug sensitivity profile of western Kenya *Plasmodium falciparum* field isolates determined by a SYBR Green I *in vitro* assay and molecular analysis. *The American Journal of Tropical Medicine and Hygiene*, *85*(1), 34–41.

Al-Musayeib, N. M., Mothana, R. A., Al-Massarani, S., Matheeussen, A., Cos, P., & Maes, L. (2012). Study of the *in vitro* antiplasmodial, antileishmanial and antitrypanosomal activities of medicinal plants from Saudi Arabia. *Molecules*, *17*(10), 11379–11390.

Alam, M. M., Sanchez-Azqueta, A., Janha, O., Flannery, E. L., Mahindra, A., Mapesa, K., Char, A. B., Sriranganadane, D., Brancucci, N. M. B., & Antonova-Koch, Y. (2019). Validation of the protein kinase PfCLK3 as a multistage cross-species malarial drug target. *Science*, *365*(6456), eaau1682.

Alano, P. (2007). *Plasmodium falciparum* gametocytes: still many secrets of a hidden life. *Molecular Microbiology*, *66*(2), 291–302.

Alano, P., Read, D., Bruce, M., Aikawa, M., Kaido, T., Tegoshi, T., Bhatti, S., Smith, D. K., Luo, C., & Hansra, S. (1995). COS cell expression cloning of Pfg377, a *Plasmodium falciparum* gametocyte antigen associated with osmiophilic bodies. *Molecular and Biochemical Parasitology*, *74*(2), 143–156.

Almela, M. J., Lozano, S., Lelièvre, J., Colmenarejo, G., Coterón, J. M., Rodrigues, J., Gonzalez, C., & Herreros, E. (2015). A new set of chemical starting points with *Plasmodium falciparum* transmission-blocking potential for antimalarial drug discovery. *PLOS ONE*, *10*(8), e0135139.

Alonso, P. L., Brown, G., Arevalo-Herrera, M., Binka, F., Chitnis, C., Collins, F., Doumbo, O. K., Greenwood, B., Hall, B. F., Levine, M. M., Mendis, K., Newman, R. D., Plowe, C. V., Rodríguez, M. H., Sinden, R., Slutsker, L., & Tanner, M. (2011). A research agenda to underpin malaria eradication. *PLoS Medicine*, *8*(1), e1000406.

Alout, H., Krajacich, B. J., Meyers, J. I., Grubaugh, N. D., Brackney, D. E., Kobylinski, K. C., Diclaro 2nd, J. W., Bolay, F. K., Fakoli, L. S., Diabaté, A., Dabiré, R. K., Bougma, R. W., & Foy, B. D. (2014). Evaluation of ivermectin mass drug administration for malaria transmission control across different West African environments. *Malaria Journal*, *13*, 417.

Aluoch, A. O. (2000). Chemistry and biological activities of *Zanthoxylum chalybeum* and *Erythrina excelsa*. University of Nairobi.

Aly, A. S. I., & Matuschewski, K. (2005). A malarial cysteine protease is necessary for *Plasmodium* sporozoite egress from oocysts. *The Journal of Experimental Medicine*, *202*(2), 225–230.

Amadi, J. A., Olago, D. O., Ong'amo, G. O., Oriaso, S. O., Nanyingi, M., Nyamongo, I. K., & Estambale, B. B. A. (2018). Sensitivity of vegetation to climate variability and its implications for malaria risk in Baringo, Kenya. *PLOS ONE*, *13*(7), e0199357.

Aminake, M. N., Schoof, S., Sologub, L., Leubner, M., Kirschner, M., Arndt, H., & Pradel, G. (2011). Thiostrepton and derivatives exhibit antimalarial and gametocytocidal activity by dually targeting parasite. *Antimicrob Agents Chemother*, *55*(4), 1338–1348.

Amoah, L. E., Kakaney, C., Kwansa-Bentum, B., & Kusi, K. A. (2015). Activity of herbal medicines on *Plasmodium falciparum* gametocytes: Implications for malaria

transmission in Ghana. *Plos One*, *10*(11), e0142587.

Amresh, G., Reddy, G. D., Rao, C. V, & Singh, P. N. (2007). Evaluation of anti-inflammatory activity of *Cissampelos pareira* root in rats. *Journal of Ethnopharmacology*, *110*(3), 526–531.

Andrade-Neto, V. F. de, Pohlit, A. M., Pinto, A. C. S., Silva, E. C. C., Nogueira, K. L., Melo, M. R. S., Henrique, M. C., Amorim, R. C. N., Silva, L. F. R., Costa, M. R. F., Nunomura, R. C. S., Nunomura, S. M., Alecrim, W. D., Alecrim, M. das G. C., Chaves, F. C. M., & Vieira, P. P. R. (2007). *In vitro* inhibition of *Plasmodium falciparum* by substances isolated from Amazonian antimalarial plants. *Memórias do Instituto Oswaldo Cruz* 102, 359–366.

Angrisano, F., Sala, K. A., Da, D. F., Liu, Y., Pei, J., Grishin, N. V, Snell, W. J., & Blagborough, A. M. (2017). Targeting the conserved fusion loop of HAP2 inhibits the transmission of *Plasmodium berghei* and *falciparum*. *Cell Reports*, *21*(10), 2868–2878.

Angrisano, F., Tan, Y., Sturm, A., Mcfadden, G. I., & Baum, J. (2012). Malaria parasite colonisation of the mosquito midgut – Placing the *Plasmodium* ookinete centre stage. *International Journal for Parasitology*, *42*(6), 519–527.

Anza, M., Haile, E., Tadesse, S., Mammo, F., & Endale, M. (2014). A coniferyl alcohol derivative from the roots of *Zanthoxylum chalybeum*. *Journal of Coastal Life Medicine*, *2*(12), 970–974.

Aqeel, A., Khursheed, A. K., Viqaruddin, A., & Sabiha, Q. (1989). Antimicrobial activity of julifloricine isolated from *Prosopis juliflora*. *Arzneimittel-Forschung*, *39*(6), 652–655.

Arai, M., Alavi, Y. I. H., Mendoza, J., Billker, O., & Sinden, R. E. (2004). Isonicotinic acid hydrazide: an anti-tuberculosis drug inhibits malarial transmission in the mosquito gut. *Experimental Parasitology*, *106*(1), 30–36.

Arama, C., & Troye-Blomberg, M. (2014). The path of malaria vaccine development: Challenges and perspectives. *Journal of Internal Medicine*, *275*(5), 456–466.

Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A.-C., Khim, N., Kim,

S., Duru, V., Bouchier, C., & Ma, L. (2014). A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature*, *505*(7481), 50–55.

Armistead, J. S., Wilson, I. B. H., van Kuppevelt, T. H., & Dinglasan, R. R. (2011). A role for heparan sulfate proteoglycans in *Plasmodium falciparum* sporozoite invasion of anopheline mosquito salivary glands. *The Biochemical Journal*, *438*(3), 475–483.

Arrighi, R. B. G., Lycett, G., Mahairaki, V., Siden-Kiamos, I., & Louis, C. (2005). Laminin and the malaria parasites journey through the mosquito midgut. *Journal of Experimental Biology*, *208*(13), 2497 LP – 2502.

Ashley, E. A., Dhorda, M., Fairhurst, R. M., Amaratunga, C., Lim, P., Suon, S., Sreng, S., Anderson, J. M., Mao, S., Sam, B., Sopha, C., Chuor, C. M., Nguon, C., Sovannaroth, S., Pukrittayakamee, S., Jittamala, P., Chotivanich, K., Chutasmit, K., Suchatsoonthorn, C., … White, N. J. (2014). Spread of Artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine*, *371*(5), 411–423.

Atkinson, S. C., Armistead, J. S., Mathias, D. K., Sandeu, M. M., Tao, D., Borhani-Dizaji, N., Tarimo, B. B., Morlais, I., Dinglasan, R. R., & Borg, N. A. (2015). The *Anopheles*-midgut APN1 structure reveals a new malaria transmission–blocking vaccine epitope. *Nature Structural & Molecular Biology*, *22*(7), 532–539.

Azebaze, A. G. B., Meyer, M., Valentin, A., Nguemfo, E. L., Fomum, Z. T., & Nkengfack, A. E. (2006). Prenylated xanthone derivatives with antiplasmodial activity from *Allanblackia monticola* STANER L.C. *Chemical and Pharmaceutical Bulletin*, *54*(1), 111–113.

Babiker, H. A., & Walliker, D. (1997). Current views on the population structure of *Plasmodium falciparum*: Implications for control. *Parasitology Today*, *13*(7), 262–267.

Baird, J. K., & Hoffman, S. L. (2004). Primaquine therapy for malaria. *Clinical Infectious Diseases*, *39*(9), 1336–1345.

Baker, D. A. (2010). Malaria gametocytogenesis. *Molecular and Biochemical*

Parasitology, *172*(2), 57–65.

Baker, D. A., Stewart, L. B., Large, J. M., Bowyer, P. W., Ansell, K. H., Jiménez-Díaz, M. B., El Bakkouri, M., Birchall, K., Dechering, K. J., Bouloc, N. S., Coombs, P. J., Whalley, D., Harding, D. J., Smiljanic-Hurley, E., Wheldon, M. C., Walker, E. M., Dessens, J. T., Lafuente, M. J., Sanz, L. M., … Osborne, S. A. (2017). A potent series targeting the malarial cGMP-dependent protein kinase clears infection and blocks transmission. *Nature Communications*, *8*(1), 430.

Bala, M., Kumar, S., Pratap, K., Verma, P. K., Padwad, Y., & Singh, B. (2017). Bioactive isoquinoline alkaloids from *Cissampelos pareira*. *Natural Product Research*, 1–6.

Balaich, J. N., Mathias, D. K., Torto, B., Jackson, B. T., Tao, D., Ebrahimi, B., & Tarimo, B. B. (2016). The nonartemisinin sesquiterpene lactones parthenin and parthenolide block *Plasmodium falciparum* sexual stage transmission. *Antimicrob Agents Chemother*, *60*(4), 2108–2117.

Baldas, J., Bick, I. R. C., Ibuka, T., Kapil, R. S., & Porter, Q. N. (1972). Mass spectrometry of bisbenzylisoquinoline alkaloids. Part I. Alkaloids derived from coclaurine units joined tail-to-tail. *Journal of the Chemical Society, Perkin Transactions 1*, *1*, 592–596.

Bansal, A., Molina-Cruz, A., Brzostowski, J., Liu, P., Luo, Y., Gunalan, K., Li, Y., Ribeiro, J. M. C., & Miller, L. H. (2018). PfCDPK1 is critical for malaria parasite gametogenesis and mosquito infection. *Proceedings of the National Academy of Sciences*, *115*(4), 774–779.

Baragaña, B., Hallyburton, I., Lee, M. C. S., Norcross, N. R., Grimaldi, R., Otto, T. D., Proto, W. R., Blagborough, A. M., Meister, S., Wirjanata, G., Ruecker, A., Upton, L. M., Abraham, T. S., Almeida, M. J., Pradhan, A., Porzelle, A., Luksch, T., Martínez, M. S., Luksch, T., … Gilbert, I. H. (2015). A novel multiple-stage antimalarial agent that inhibits protein synthesis. *Nature*, *522*(7556), 315–320.

Batista, R., De Jesus Silva Júnior, A., & De Oliveira, A. B. (2009). Plant-derived antimalarial agents: New leads and efficient phytomedicines. Part II. Non-alkaloidal

natural products. *Molecules* 14, 8.

Batista, R., Santana, C. C., Azevedo-Santos, A. V., Suarez-Fontes, A. M., Ferraz, J. L. de A. A., Silva, L. A. M., & Vannier-Santos, M. A. (2018a). *In vivo* antimalarial extracts and constituents of *Prosopis juliflora* (Fabaceae). *Journal of Functional Foods*, *44*, 74–78.

Baton, L A, & Ranford-Cartwright, L. C. (2004). *Plasmodium falciparum* ookinete invasion of the midgut epithelium of *Anopheles stephensi* is consistent with the Time Bomb model. *Parasitology*, *129*(6), 663–676.

Baton, Luke A, Garver, L., Xi, Z., & Dimopoulos, G. (2008). Functional genomics studies on the innate immunity of disease vectors. *Insect Science*, *15*(1), 15–27.

Baton, Luke A, & Ranford-Cartwright, L. C. (2005). How do malaria ookinetes cross the mosquito midgut wall? *Trends in Parasitology*, *21*(1), 22–28.

Bayih, A. G., Getnet, G., Alemu, A., Getie, S., Mohon, A. N., & Pillai, D. R. (2016). A unique *Plasmodium falciparum* K13 gene mutation in Northwest Ethiopia. *The American Journal of Tropical Medicine and Hygiene*, *94*(1), 132–135.

Bechtsi, D. P., & Waters, A. P. (2017). Genomics and epigenetics of sexual commitment in *Plasmodium*. *International Journal for Parasitology*, *47*(7), 425–434.

Beier, J. C. (1998). Malaria parasite development in mosquitoes. *Annual Review of Entomology*, *43*(1), 519–543.

Beier, J. C., Killeen, G. F., & Githure, J. I. (1999). Entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. *The American Journal of Tropical Medicine and Hygiene*, *61*(1), 109–113.

Bell, A. (1998). Microtubule inhibitors as potential antimalarial agents. *Parasitology Today (Personal Ed.)*, *14*(6), 234—240.

Bennink, S., Kiesow, M. J., & Pradel, G. (2016). The development of malaria parasites in the mosquito midgut. *Cellular Microbiology*, *18*(7), 905–918.

Bennink, S., von Bohl, A., Ngwa, C. J., Henschel, L., Kuehn, A., Pilch, N., Weißbach, T., Rosinski, A. N., Scheuermayer, M., Repnik, U., Przyborski, J. M., Minns, A. M., Orchard, L. M., Griffiths, G., Lindner, S. E., Llinás, M., & Pradel, G. (2018). A

seven-helix protein constitutes stress granules crucial for regulating translation during human-to-mosquito transmission of *Plasmodium falciparum*. *PLOS Pathogens*, *14*(8), e1007249.

Beri, D., Balan, B., Chaubey, S., Subramaniam, S., Surendra, B., & Tatu, U. (2017). A disrupted transsulphuration pathway results in accumulation of redox metabolites and induction of gametocytogenesis in malaria. *Scientific Reports*, *7*, 40213.

Bero, J., Frédérich, M., & Quetin-Leclercq, J. (2009). Antimalarial compounds isolated from plants used in traditional medicine. *Journal of Pharmacy and Pharmacology*, *61*(11), 1401–1433.

Bertani, S., Houël, E., Stien, D., Chevolot, L., Jullian, V., Garavito, G., Bourdy, G., & Deharo, E. (2006). Simalikalactone D is responsible for the antimalarial properties of an amazonian traditional remedy made with *Quassia amara* L. (Simaroubaceae). *Journal of Ethnopharmacology*, *108*(1), 155–157.

Beshir, K. B., Sutherland, C. J., Sawa, P., Drakeley, C. J., Okell, L., Mweresa, C. K., Omar, S. A., Shekalaghe, S. A., Kaur, H., & Ndaro, A. (2013). Residual *Plasmodium falciparum* parasitemia in Kenyan children after artemisinin-combination therapy is associated with increased transmission to mosquitoes and parasite recurrence. *The Journal of Infectious Diseases*, *208*(12), 2017–2024.

Bhagavathula, A. S., Elnour, A. A., & Shehab, A. (2016). Alternatives to currently used antimalarial drugs: in search of a magic bullet. *Infectious Diseases of Poverty*, *5*(1), 103.

Billker, O, Lindo, V., Panico, M., Etienne, A. E., Paxton, T., Dell, A., Rogers, M., Sinden, R. E., & Morris, H. R. (1998). Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito. *Nature*, *392*(6673), 289–292.

Billker, O., Dechamps, S., Tewari, R., Wenig, G., Franke-Fayard, B., & Brinkmann, V. (2004). Calcium and a calcium-dependent protein kinase regulate gamete formation and mosquito transmission in a malaria parasite. *Cell*, *117*(4), 503–514.

Billker, O., Lourido, S., & Sibley, L. D. (2009). Calcium-dependent signaling and

kinases in apicomplexan parasites. *Cell Host & Microbe*, *5*(6), 612–622.

Billker, O., Shaw, M. K., Jones, I. A. N. W., Ley, S. V, Luntz, A. J. M., & Sinden, R. E. (2002). Azadirachtin disrupts formation of organised microtubule arrays during microgametogenesis of *Plasmodium berghei*. *Journal of Eukaryotic Microbiology*, *49*(6), 489–497.

Birkett, A. J. (2016). Status of vaccine research and development of vaccines for malaria. *Vaccine*, *34*(26), 2915–2920.

Birkholtz, L.-M., Coetzer, T. L., Mancama, D., Leroy, D., & Alano, P. (2016). Discovering new transmission-blocking antimalarial compounds: Challenges and opportunities. *Trends in Parasitology*, *32*(9), 669–681.

Blagborough, A. M., Delves, M. J., Ramakrishnan, C., Lal, K., Butcher, G., & Sinden, R. E. (2013). Assessing transmission blockade in *Plasmodium spp.* - *Malaria: Methods and Protocols* (R. Ménard (Ed.); pp. 577–600).

Blandin, S., Shiao, S.-H., Moita, L. F., Janse, C. J., Waters, A. P., Kafatos, F. C., & Levashina, E. A. (2004). Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell*, *116*(5), 661–670.

Blasco, B., Leroy, D., & Fidock, D. A. (2017). Antimalarial drug resistance: linking *Plasmodium falciparum* parasite biology to the clinic. *Nature Medicine*, *23*(8), 917.

Bobenchik, A. M., Witola, W. H., Augagneur, Y., Nic Lochlainn, L., Garg, A., Pachikara, N., Choi, J.-Y., Zhao, Y. O., Usmani-Brown, S., Lee, A., Adjalley, S. H., Samanta, S., Fidock, D. A., Voelker, D. R., Fikrig, E., & Ben Mamoun, C. (2013). *Plasmodium falciparum* phosphoethanolamine methyltransferase is essential for malaria transmission. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(45), 18262–18267.

Bohbot, J. D., & Dickens, J. C. (2012). Selectivity of odorant receptors in insects. *Frontiers in Cellular Neuroscience*, *6*, 29.

Boissière, A., Tchioffo, M. T., Bachar, D., Abate, L., Marie, A., Nsango, S. E., Shahbazkia, H. R., Awono-Ambene, P. H., Levashina, E. A., Christen, R., & Morlais, I. (2012). Midgut microbiota of the malaria mosquito vector *Anopheles*

gambiae and interactions with *Plasmodium falciparum* infection. *PLOS Pathogens*, *8*(5), e1002742.

Bompard, A., Da, D. F., Yerbanga, R. S., Biswas, S., Kapulu, M., Bousema, T., Lefèvre, T., Cohuet, A., & Churcher, T. S. (2017). Evaluation of two lead malaria transmission blocking vaccine candidate antibodies in natural parasite-vector combinations. Scientific Reports 7, 6766.

Bousema, T., Sutherland, C. J., Churcher, T. S., Mulder, B., Gouagna, L. C., Riley, E. M., Targett, G. A. T., & Drakeley, C. J. (2011). Human immune responses that reduce the transmission of *Plasmodium falciparum* in African populations. *International Journal for Parasitology*, *41*(3–4), 293–300.

Bozdech, Z., Llinás, M., Pulliam, B. L., Wong, E. D., Zhu, J., & DeRisi, J. L. (2003). The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. *PLoS Biology*, *1*(1), E5–E5.

Brancucci, N. M. B., Bertschi, N. L., Zhu, L., Niederwieser, I., Chin, W. H., Wampfler, R., Freymond, C., Rottmann, M., Felger, I., Bozdech, Z., & Voss, T. S. (2014). Heterochromatin Protein 1 secures survival and transmission of malaria parasites. *Cell Host & Microbe*, *16*(2), 165–176.

Brancucci, N. M. B., Gerdt, J. P., Wang, C., De Niz, M., Philip, N., Adapa, S. R., Zhang, M., Hitz, E., Niederwieser, I., & Boltryk, S. D. (2017). Lysophosphatidylcholine regulates sexual stage differentiation in the human malaria parasite *Plasmodium falciparum*. *Cell*, *171*(7), 1532–1544.

Bridgford, J. L., Xie, S. C., Cobbold, S. A., Pasaje, C. F. A., Herrmann, S., Yang, T., Gillett, D. L., Dick, L. R., Ralph, S. A., Dogovski, C., Spillman, N. J., & Tilley, L. (2018). Artemisinin kills malaria parasites by damaging proteins and inhibiting the proteasome. *Nature Communications*, *9*, 3801.

Bringmann, G., Mutanyatta-Comar, J., Knauer, M., & Abegaz, B. M. (2008). Knipholone and related 4-phenylanthraquinones: structurally, pharmacologically, and biosynthetically remarkable natural products. *Natural Product Reports*, *25*(4), 696–718.

Bruce, M. C., Carter, R. N., Nakamura, K., Aikawa, M., & Carter, R. (1994). Cellular location and temporal expression of the *Plasmodium falciparum* sexual stage antigen Pfs16. *Molecular and Biochemical Parasitology*, *65*(1), 11–22.

Bunnik, E. M., Cook, K. B., Varoquaux, N., Batugedara, G., Prudhomme, J., Cort, A., Shi, L., Andolina, C., Ross, L. S., Brady, D., Fidock, D. A., Nosten, F., Tewari, R., Sinnis, P., Ay, F., Vert, J.-P., Noble, W. S., & Le Roch, K. G. (2018). Changes in genome organization of parasite-specific gene families during the *Plasmodium* transmission stages. *Nature Communications*, *9*(1), 1910.

Burrows, J. N., Duparc, S., Gutteridge, W. E., Hooft van Huijsduijnen, R., Kaszubska, W., Macintyre, F., Mazzuri, S., Möhrle, J. J., & Wells, T. N. C. (2017). New developments in anti-malarial target candidate and product profiles. *Malaria Journal*, *16*(1), 26.

Busula, A. O., Bousema, T., Mweresa, C. K., & Masiga, D. (2017). Gametocytemia and attractiveness of *Plasmodium falciparum* – infected Kenyan children to *Anopheles gambiae* mosquitoes. *The Journal of Infectious Diseases*, *216*, 291–295.

Busula, A. O., Bousema, T., Mweresa, C. K., Masiga, D., Logan, J. G., Sauerwein, R. W., Verhulst, N. O., Takken, W., & de Boer, J. G. (2017). Gametocytemia and attractiveness of Plasmodium falciparum–infected Kenyan children to *Anopheles gambiae* mosquitoes. *The Journal of Infectious Diseases*, *216*(3), 291–295.

Campell, W.C., Fisher, M.H., Stapley, E.O., Albers-Schonberg, G., & Jacob, T.A. (1983). Ivermectin: A potent new antiparasitic agent. *Science*, *221*(4613), 823–828.

Cachet, N., Hoakwie, F., Bertani, S., Bourdy, G., Deharo, E., Stien, D., Houel, E., Gornitzka, H., Fillaux, J., Chevalley, S., Valentin, A., & Jullian, V. (2009). Antimalarial activity of Simalikalactone E, a new quassinoid from *Quassia amara* L. (Simaroubaceae). *Antimicrobial Agents and Chemotherapy*, *53*(10), 4393 – 4398.

Calderón, J., Carlos, C., Rosaura, R., Federico, G.-G., R, S. J., R, S. J., Laura, L., Eduardo, A., & Isao, K. (2001). Acetylcholinesterase and insect growth inhibitory activities of *Gutierrezia microcephala* on Fall Army worm *Spodoptera frugiperda*

J. E. Smith. In *Zeitschrift für Naturforschung C* 56, 382.

Campbell, W. C. (2016). Lessons from the history of ivermectin and other antiparasitic agents. *Annual Review of Animal Biosciences*, *4*, 1–14.

Carroll, S. P., & Loye, J. (2006). PMD, a registered botanical mosquito repellent with Deet-like efficacy. *Journal of the American Mosquito Control Association*, *22*(3), 507–514.

Carter, R., & Mendis, K. N. (2002). Evolutionary and historical aspects of the burden of malaria. *Clinical Microbiology Reviews*, *15*(4), 564–594.

Carter, R., Mendis, K. N., Miller, L. H., Molineaux, L., & Saul, A. (2000). Malaria transmission-blocking vaccines—how can their development be supported? *Nature Medicine*, *6*(3), 241–244.

Carter, V., Nacer, A. M. L., Underhill, A., Sinden, R. E., & Hurd, H. (2007). Minimum requirements for ookinete to oocyst transformation in *Plasmodium*. *International Journal for Parasitology*, *37*(11), 1221–1232.

Chaal, B. K., Gupta, A. P., Wastuwidyaningtyas, B. D., Luah, Y.-H., & Bozdech, Z. (2010). Histone deacetylases play a major role in the transcriptional regulation of the *Plasmodium falciparum* life cycle. *PLOS Pathogens*, *6*(1), e1000737.

Chaccour, C. J., Kobylinski, K. C., Bassat, Q., Bousema, T., Drakeley, C., Alonso, P., & Foy, B. D. (2013). Ivermectin to reduce malaria transmission: a research agenda for a promising new tool for elimination. *Malaria Journal*, *12*(1), 153.

Chaccour, C., & Killeen, G. F. (2016). Mind the gap: residual malaria transmission, veterinary endectocides and livestock as targets for malaria vector control. *Malaria Journal*, *15*(1), 24.

Chagonda, L., Gundidza, M., Wijesuria, A. M. S., & Mavi, S. (1994). Composition of the leaf oil of *Zanthoxylum chalybeum* Thunb. *Journal of Essential Oil Research*, *6*(6), 561–563.

Charman, S. A., Arbe-Barnes, S., Bathurst, I. C., Brun, R., Campbell, M., Charman, W. N., Chiu, F. C. K., Chollet, J., Craft, J. C., Creek, D. J., Dong, Y., Matile, H., Maurer, M., Morizzi, J., Nguyen, T., Papastogiannidis, P., Scheurer, C.,

Shackleford, D. M., Sriraghavan, K., … Vennerstrom, J. L. (2011). Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria. *Proceedings of the National Academy of Sciences*, *108*(11), 4400 – 4405.

Chaubey, S., Grover, M., & Tatu, U. (2014). Endoplasmic reticulum stress triggers gametocytogenesis in the malaria parasite. *Journal of Biological Chemistry*, *289*(24), 16662–16674.

Chea, A., Bun, S.-S., Azas, N., Gasquet, M., Bory, S., Ollivier, E., & Elias, R. (2010). Antiplasmodial activity of three bisbenzylisoquinoline alkaloids from the tuber of *Stephania rotunda*. *Natural Product Research*, *24*(18), 1766–1770.

Cheng, Q., Kyle, D. E., & Gatton, M. L. (2012). Artemisinin resistance in *Plasmodium falciparum*: A process linked to dormancy? *International Journal for Parasitology: Drugs and Drug Resistance*, *2*, 249–255.

Choudhary, M. I., Nawaz, S. A., Azim, M. K., Ghayur, M. N., Lodhi, M. A., Jalil, S., Khalid, A., Ahmed, A., Rode, B. M., & Ahmad, V. U. (2005). Juliflorine: a potent natural peripheral anionic-site-binding inhibitor of acetylcholinesterase with calcium-channel blocking potential, a leading candidate for Alzheimer's disease therapy. *Biochemical and Biophysical Research Communications*, *332*(4), 1171–1179.

Christophides, G. K., Vlachou, D., & Kafatos, F. C. (2004). Comparative and functional genomics of the innate immune system in the malaria vector *Anopheles gambiae*. *Immunological Reviews*, *198*(1), 127–148.

Chugh, M., Gulati, B. R., & Gakhar, S. K. (2010). Monoclonal antibodies AC-43 and AC-29 disrupt *Plasmodium vivax* development in the Indian malaria vector *Anopheles culicifacies* (Diptera: culicidae). *Journal of Biosciences*, *35*(1), 87–94.

Chukwujekwu, J. C., Smith, P., Coombes, P. H., Mulholland, D. A., & Van Staden, J. (2005). Antiplasmodial diterpenoid from the leaves of *Hyptis suaveolens*. *Journal of Ethnopharmacology*, *102*(2), 295–297.

Chung, I.-M., Kim, M.-Y., & Moon, H.-I. (2008). Antiplasmodial activity of

sesquiterpene lactone from *Carpesium rosulatum* in mice. *Parasitology Research*, *103*(2), 341–344.

Churcher, T. S., Sinden, R. E., Edwards, N. J., Poulton, I. D., Rampling, W., Brock, P. M., Griffin, J. T., Upton, L. M., Zakutansky, E., Sala, K. A., Angrisano, F., Hill, A. V. S., & Blagborough, M. (2017). Probability of transmission of malaria from mosquito to human is regulated by mosquito parasite density in naïve and vaccinated hosts. *PLoS Pathogens*, *13(1)*, e1006108.

Churcher, T. S., Trape, J.-F., & Cohuet, A. (2015). Human-to-mosquito transmission efficiency increases as malaria is controlled. *Nature Communications*, *6*, 6054.

Cirimotich, C. M., Ramirez, J. L., & Dimopoulos, G. (2011). Native microbiota shape insect vector competence for human pathogens. *Cell Host & Microbe*, *10*(4), 307–310.

Clayton, A. M., Cirimotich, C. M., Dong, Y., & Dimopoulos, G. (2013). Caudal is a negative regulator of the *Anopheles* IMD Pathway that controls resistance to *Plasmodium falciparum* infection. *Developmental & Comparative Immunology*, *39*(4), 323–332.

Coelho, C. H., Doritchamou, J. Y. A., Zaidi, I., & Duffy, P. E. (2017). Advances in malaria vaccine development: report from the 2017 malaria vaccine symposium. *NPJ Vaccines*, *2*, 34.

Coertzen, D., Reader, J., van der Watt, M., Nondaba, S. H., Gibhard, L., Wiesner, L., Smith, P., Alessandro, S., Taramelli, D., Wong, H. N., du Preez, J. L., Wu, R. W. K., Birkholtz, L.-M., & Haynes, R. K. (2018). Artemisone and Artemiside are potent panreactive antimalarial agents that also synergize redox imbalance in *Plasmodium falciparum* transmissible gametocyte stages. *Antimicrobial Agents and Chemotherapy*, *62*(8), e02214-17.

Coetzee, N., von Grüning, H., Opperman, D., van der Watt, M., Reader, J., & Birkholtz, L.-M. (2020). Epigenetic inhibitors target multiple stages of *Plasmodium falciparum* parasites. *Scientific Reports*, *10*(1), 2355.

Cogswell, F. B., Krogstad, D. J., De, D., & Krogstad, F. M. (1996). Aminoquinolines

that circumvent resistance in *Plasmodium falciparum in vitro*. *The American Journal of Tropical Medicine and Hygiene*, *55*(6), 579–583.

Cohen, E. (1981). Acetylcholinesterase activity in an *Aedes aegypti* cell line. *Experientia*, *37*(4), 429–431.

Cohuet, A., Harris, C., Robert, V., & Fontenille, D. (2010). Evolutionary forces on *Anopheles*: what makes a malaria vector? *Trends in Parasitology*, *26*(3), 130–136.

Coleman, B. I., Skillman, K. M., Jiang, R. H. Y., Childs, L. M., Altenhofen, L. M., Ganter, M., Leung, Y., Goldowitz, I., Kafsack, B. F. C., Marti, M., Llinás, M., Buckee, C. O., & Duraisingh, M. T. (2014). A *Plasmodium falciparum* histone deacetylase regulates antigenic variation and gametocyte conversion. *Cell Host & Microbe*, *16*(2), 177–186.

Coleman, R. E., Kumpitak, C., Ponlawat, A., Maneechai, N., Phunkitchar, V., Rachapaew, N., Zollner, G., & Sattabongkot, J. (2004). Infectivity of asymptomatic *Plasmodium*-Infected human populations to *Anopheles dirus* mosquitoes in Western Thailand. *Journal of Medical Entomology*, *41*(2), 201–208.

Cooke, M. K., Kahindi, S. C., Oriango, R. M., Owaga, C., Ayoma, E., Mabuka, D., Nyangau, D., Abel, L., Atieno, E., Awuor, S., Drakeley, C., Cox, J., & Stevenson, J. (2015). "A bite before bed": exposure to malaria vectors outside the times of net use in the highlands of western Kenya. *Malaria Journal*, *14*, 259.

Coulibaly, B., Zoungrana, A., Mockenhaupt, F. P., Schirmer, R. H., Klose, C., Mansmann, U., Meissner, P. E., & Müller, O. (2009). Strong gametocytocidal effect of methylene blue-based combination therapy against *falciparum* malaria: A randomised controlled trial. *PLOS ONE*, *4*(5), e5318.

Cowell, A. N., Istvan, E. S., Lukens, A. K., Gomez-Lorenzo, M. G., Vanaerschot, M., Sakata-Kato, T., Flannery, E. L., Magistrado, P., Owen, E., & Abraham, M. (2018). Mapping the malaria parasite druggable genome by using *in vitro* evolution and chemogenomics. *Science*, *359*(6372), 191–199.

Cox, F. E. G. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites & Vectors*, *3*(1), 5.

Cragg, G. M., & Newman, D. J. (2013). Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1830*(6), 3670–3695.

Crompton, P. D., Pierce, S. K., & Miller, L. H. (2010). Advances and challenges in malaria vaccine development. *The Journal of Clinical Investigation*, *120*(12), 4168–4178.

Cupp, E. W., Sauerbrey, M., & Richards, F. (2011). Elimination of human onchocerciasis: history of progress and current feasibility using ivermectin (Mectizan®) monotherapy. *Acta Tropica*, *120*, S100–S108.

da Silva Costa, M., de Paula, S. O., Martins, G. F., Zanuncio, J. C., Santana, A. E. G., & Serrão, J. E. (2016). Multiple modes of action of the squamocin in the midgut cells of *Aedes aegypti* larvae. *PLoS ONE*, *11*(8), e0160928.

Dahiya, N., Chianese, G., Abay, S. M., Taglialatela-Scafati, O., Esposito, F., Lupidi, G., Bramucci, M., Quassinti, L., Christophides, G., Habluetzel, A., & Lucantoni, L. (2016). *In vitro* and *ex vivo* activity of an *Azadirachta indica* A.Juss. seed kernel extract on early sporogonic development of *Plasmodium* in comparison with azadirachtin A, its most abundant constituent. *Phytomedicine*, *23*(14), 1743–1752.

Dahl, E. L., & Rosenthal, P. J. (2007). Multiple antibiotics exert delayed effects against the *Plasmodium falciparum* apicoplast. *Antimicrobial Agents and Chemotherapy*, *51*(10), 3485–3490.

Das, S., Bhatanagar, S., Morrisey, J. M., Daly, T. M., Burns Jr, J. M., Coppens, I., & Vaidya, A. B. (2016). Na+ influx induced by new antimalarials causes rapid alterations in the cholesterol content and morphology of *Plasmodium falciparum*. *PLOS Pathogens*, *12*(5), e1005647.

Day, K. P., Karamalis, F., Thompson, J., Barnes, D. A., Peterson, C., Brown, H., Brown, G. V, & Kemp, D. J. (1993). Genes necessary for expression of a virulence determinant and for transmission of *Plasmodium falciparum* are located on a 0.3-megabase region of chromosome 9. *Proceedings of the National Academy of Sciences*, *90*(17), 8292–8296.

de Almeida Oliveira, G., Lieberman, J., & Barillas-Mury, C. (2012). Epithelial nitration by a peroxidase/NOX5 system mediates mosquito antiplasmodial immunity. *Science*, *335*(6070), 856–859.

De Koning-Ward, T. F., Olivieri, A., Bertuccini, L., Hood, A., Silvestrini, F., Charvalias, K., Berzosa Díaz, P., Camarda, G., McElwain, T. F., Papenfuss, T., Healer, J., Baldassarri, L., Crabb, B. S., Alano, P., & Ranford-Cartwright, L. C. (2008). The role of osmiophilic bodies and Pfg377 expression in female gametocyte emergence and mosquito infectivity in the human malaria parasite *Plasmodium falciparum*. *Molecular Microbiology*, *67*(2), 278–290.

De Moraes, C. M., Wanjiku, C., Stanczyk, N. M., Pulido, H., Sims, J. W., Betz, H. S., Read, A. F., Torto, B., & Mescher, M. C. (2018). Volatile biomarkers of symptomatic and asymptomatic malaria infection in humans. *Proceedings of the National Academy of Sciences*, *115*(22), 5780–5785.

De Niz, M., Meibalan, E., Mejia, P., Ma, S., Brancucci, N. M. B., Agop-Nersesian, C., Mandt, R., Ngotho, P., Hughes, K. R., Waters, A. P., Huttenhower, C., Mitchell, J. R., Martinelli, R., Frischknecht, F., Seydel, K. B., Taylor, T., Milner, D., Heussler, V. T., & Marti, M. (2018). *Plasmodium* gametocytes display homing and vascular transmigration in the host bone marrow. *Science Advances*, *4*(5), eaat3775.

de Villiers, K. A., & Egan, T. J. (2009). Recent advances in the discovery of haem-targeting drugs for malaria and schistosomiasis. *Molecules*, *14*(8), 2868–2887.

Dearnley, M., Chu, T., Zhang, Y., Looker, O., Huang, C., Klonis, N., Yeoman, J., Kenny, S., Arora, M., Osborne, J. M., Chandramohanadas, R., Zhang, S., Dixon, M. W. A., & Tilley, L. (2016). Reversible host cell remodeling underpins deformability changes in malaria parasite sexual blood stages. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(17), 4800–4805.

Debboun, M., Frances, S. P., & Strickman, D. (2006). *Insect repellents: principles, methods, and uses*. CRC press.

Deligianni, E., Morgan, R. N., Bertuccini, L., Kooij, T. W. A., Laforge, A., Nahar, C., Poulakakis, N., Schüler, H., Louis, C., & Matuschewski, K. (2011). Critical role for

a stage-specific actin in male exflagellation of the malaria parasite. *Cellular Microbiology*, *13*(11), 1714–1730.

Delves, M. J., Angrisano, F., & Blagborough, A. M. (2018). Antimalarial transmission-blocking interventions: Past, present, and future. *Trends in Parasitology*, *34*(9), 735–746.

Dembo, E. G., Abay, S. M., Dahiya, N., Ogboi, J. S., Christophides, G. K., Lupidi, G., Chianese, G., Lucantoni, L., & Habluetzel, A. (2015). Impact of repeated NeemAzal®-treated blood meals on the fitness of *Anopheles stephensi* mosquitoes. *Parasites & Vectors*, *8*(1), 1–13.

Demissew, A., Balkew, M., & Girma, M. (2016). Larvicidal activities of chinaberry, neem and *Bacillus thuringiensis* israelensis (Bti) to an insecticide resistant population of *Anopheles arabiensis* from Tolay, Southwest Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*, *6*(7), 554–561.

Desgrouas, C., Chapus, C., Desplans, J., Travaille, C., Pascual, A., Baghdikian, B., Ollivier, E., Parzy, D., & Taudon, N. (2014). *In vitro* antiplasmodial activity of cepharanthine. *Malar J*, *13*, 327.

Dessens, J. T., Beetsma, A. L., Dimopoulos, G., Wengelnik, K., Crisanti, A., Kafatos, F. C., & Sinden, R. E. (1999). CTRP is essential for mosquito infection by malaria ookinetes. *The EMBO Journal*, *18*(22), 6221–6227.

Dettrakul, S., Surerum, S., Rajviroongit, S., & Kittakoop, P. (2009). Biomimetic transformation and biological activities of globiferin, a terpenoid benzoquinone from *Cordia globifera*. *Journal of Natural Products*, *72*(5), 861–865.

Dhawan, R., Gupta, K., Kajla, M., Kakani, P., Choudhury, T. P., Kumar, S., Kumar, V., & Gupta, L. (2017). Apolipophorin-III acts as a positive regulator of *Plasmodium* development in *Anopheles stephensi*. *Frontiers in Physiology*, *8*, 185.

Diagana, T. T. (2015). Supporting malaria elimination with 21st century antimalarial agent drug discovery. *Drug Discovery Today*, *20*(10), 1265–1270.

Dicko, A., Roh, M. E., Diawara, H., Mahamar, A., Soumare, H. M., Lanke, K., Bradley, J., Sanogo, K., Kone, D. T., Diarra, K., Keita, S., Issiaka, D., Traore, S. F.,

McCulloch, C., Stone, W. J. R., Hwang, J., Müller, O., Brown, J. M., Srinivasan, V., … Bousema, T. (2018). Efficacy and safety of primaquine and methylene blue for prevention of *Plasmodium falciparum* transmission in Mali: a phase 2, single-blind, randomised controlled trial. *The Lancet Infectious Diseases*, *18*(6), 627–639.

Dinan, L. (2001). Phytoecdysteroids: biological aspects. *Phytochemistry*, *57*(3), 325–339.

Dinglasan, R. R., Alaganan, A., Ghosh, A. K., Saito, A., Kuppevelt, T. H. Van, & Jacobs-lorena, M. (2007). *Plasmodium falciparum* ookinetes require mosquito midgut chondroitin sulfate proteoglycans for cell invasion. *Proceedings of the National Academy of Sciences*, *104(40)*, 15882 - 15887

Dinglasan, R. R., Kalume, D. E., Kanzok, S. M., Ghosh, A. K., Muratova, O., Pandey, A., & Jacobs-lorena, M. (2007). Disruption of *Plasmodium falciparum* development by antibodies against a conserved mosquito midgut antigen. *Proceedings of the National Academy of Sciences*, *104(33)*, 13461 - 13466

Doerig, C., Rayner, J. C., Scherf, A., & Tobin, A. B. (2015). Post-translational protein modifications in malaria parasites. *Nature Reviews Microbiology*, *13*(3), 160–172.

Dogovski, C., Xie, S. C., Burgio, G., Bridgford, J., Mok, S., McCaw, J. M., Chotivanich, K., Kenny, S., Gnädig, N., Straimer, J., Bozdech, Z., Fidock, D. A., Simpson, J. A., Dondorp, A. M., Foote, S., Klonis, N., & Tilley, L. (2015). Targeting the cell Stress response of *Plasmodium falciparum* to overcome artemisinin resistance. *PLOS Biology*, *13*(4), e1002132.

Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phyo, A. P., Tarning, J., Lwin, K. M., Ariey, F., Hanpithakpong, W., Lee, S. J., Ringwald, P., Silamut, K., Imwong, M., Chotivanich, K., Lim, P., Herdman, T., An, S. S., Yeung, S., Singhasivanon, P., … White, N. J. (2009). Artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine*, *361*(5), 455–467.

Dondorp, A. M., Smithuis, F. M., Woodrow, C., & von Seidlein, L. (2017). How to contain artemisinin-and multidrug-resistant *falciparum* malaria. *Trends in Parasitology*, *33*(5), 353–363.

Dong, Y, Manfredini, F., & Dimopoulos, G. (2009). Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathogens*, *5*(5), e1000423.

Dong, Yuemei, Simões, M. L., Marois, E., & Dimopoulos, G. (2018). CRISPR/Cas9 -mediated gene knockout of *Anopheles gambiae* FREP1 suppresses malaria parasite infection. *PLoS Pathogens*, *14*(3), e1006898.

dos Santos, T. E., Pereira, L. M., da Silva, F. C., Souza-Neta, C. L., Geris, R., Martins, D., Santana, E. A., Barbosa, C. L., Silva, G. H., Freitas, C. G., Figueiredo, P. M., de Oliveira, F. F., & Batista, R. (2013). Antibacterial activity of the alkaloid-enriched extract from *Prosopis juliflora* pods and its influence on *in vitro* ruminal digestion. *International Journal of Molecular Sciences 14*, 4.

Dou, D., Park, J. G., Rana, S., Madden, B. J., Jiang, H., & Pang, Y.-P. (2013). Novel selective and irreversible mosquito acetylcholinesterase inhibitors for controlling malaria and other mosquito-borne Diseases. *Scientific Reports*, *3*, 1068.

Drakeley, C. J., Bousema, J. T., Akim, N. I. J., Teelen, K., Roeffen, W., Lensen, A. H., Bolmer, M., Eling, W., & Sauerwein, R. W. (2006). Transmission-reducing immunity is inversely related to age in *Plasmodium falciparum* gametocyte carriers. *Parasite Immunology*, *28*(5), 185–190.

Draper, S. J., Sack, B. K., King, C. R., Nielsen, C. M., Rayner, J. C., Higgins, M. K., Long, C. A., & Seder, R. A. (2018). Malaria Vaccines: Recent advances and new horizons. *Cell Host & Microbe*, *24*(1), 43–56.

Du, Y., Valenciano, A. L., Dai, Y., Zheng, Y., Zhang, F., Zhang, Y., Clement, J., Goetz, M., Kingston, D. G. I., & Cassera, M. B. (2020). Anibamine and its analogues: Potent antiplasmodial agents from *Aniba citrifolia*. *Journal of Natural Products 83(3)*, 569-577

Dubar, F., Khalife, J., Brocard, J., Dive, D., & Biot, C. (2008). Ferroquine, an ingenious antimalarial drug–thoughts on the mechanism of action. *Molecules*, *13*(11), 2900–2907.

Duffy, P. E., & Kaslow, D. C. (1997). A novel malaria protein, Pfs28, and Pfs25 are

genetically linked and synergistic as *falciparum* malaria transmission-blocking vaccines. *Infection and Immunity*, *65*(3), 1109–1113.

Ecker, A., Pinto, S. B., Baker, K. W., Kafatos, F. C., & Sinden, R. E. (2007). *Plasmodium berghei*: *Plasmodium* perforin-like protein 5 is required for mosquito midgut invasion in *Anopheles stephensi*. *Experimental Parasitology*, *116*(4), 504–508.

Eckstein-Ludwig, U., Webb, R. J., Van Goethem, I. D. A., East, J. M., Lee, A. G., Kimura, M., O'neill, P. M., Bray, P. G., Ward, S. A., & Krishna, S. (2003). Artemisinins target the SERCA of *Plasmodium falciparum*. *Nature*, *424*(6951), 957.

Eksi, S., Haile, Y., Furuya, T., Ma, L., Su, X., & Williamson, K. C. (2005). Identification of a subtelomeric gene family expressed during the asexual–sexual stage transition in *Plasmodium falciparum*. *Molecular and Biochemical Parasitology*, *143*(1), 90–99.

Eksi, S., Morahan, B. J., Haile, Y., Furuya, T., Jiang, H., Ali, O., Xu, H., Kiattibutr, K., Suri, A., Czesny, B., Adeyemo, A., Myers, T. G., Sattabongkot, J., Su, X., & Williamson, K. C. (2012). *Plasmodium falciparum* gametocyte development 1 (Pfgdv1) and gametocytogenesis early gene identification and commitment to sexual development. *PLOS Pathogens*, *8*(10), e1002964.

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88–95.

Engeu, O. P., Tumusiime, R., Agwaya, M., Mugisha, G., Nambatya Kyeyune, G., Galiwango, B., & Waako, P. (2008). Repeat-dose effects of *Zanthoxylum chalybeum* root bark extract: a traditional medicinal plant used for various diseases in Uganda. *International Journal of Histology and Cytology*, *7*(8), 1–5.

Eriksen, J., Mwankusye, S., Mduma, S., Veiga, M. I., Kitua, A., Tomson, G., Petzold, M. G., Swedberg, G., Gustafsson, L. L., & Warsame, M. (2008). Antimalarial resistance and DHFR/DHPS genotypes of *Plasmodium falciparum* three years after

introduction of sulfadoxine–pyrimethamine and amodiaquine in rural Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *102*(2), 137–142.

Fairhurst, R. M., & Dondorp, A. M. (2016). Artemisinin-resistant *Plasmodium falciparum* malaria. *Emerging Infections 10*, 409–429.

Fennell, B. J., Naughton, J. A., Dempsey, E., & Bell, A. (2006). Cellular and molecular actions of dinitroaniline and phosphorothioamidate herbicides on *Plasmodium falciparum*: Tubulin as a specific antimalarial target. *Molecular and Biochemical Parasitology*, *145*(2), 226–238.

Ferguson, D. J. P., Balaban, A. E., Patzewitz, E.-M., Wall, R. J., Hopp, C. S., Poulin, B., Mohmmed, A., Malhotra, P., Coppi, A., Sinnis, P., & Tewari, R. (2014). The repeat region of the circumsporozoite protein is critical for sporozoite formation and maturation in *Plasmodium*. *PloS One*, *9*(12), e113923–e113923.

Fernandez, L. S., Buchanan, M. S., Carroll, A. R., Feng, Y. J., Quinn, R. J., & Avery, V. M. (2009). Flinderoles A−C: Antimalarial bis-indole alkaloids from *Flindersia* Species. *Organic Letters*, *11*(2), 329–332.

Ferrer, P., Tripathi, A. K., Clark, M. A., Hand, C. C., Young, H., Jr, R., & Sullivan, D. J. (2012). Antimalarial iron chelator , FBS0701 , shows asexual and gametocyte *Plasmodium falciparum* activity and single oral dose cure in a murine malaria model. *PLoS ONE*, *7(5)*, e0037171.

Ferrer, P., Vega-rodriguez, J., Tripathi, A. K., Jacobs-lorena, M., & Sullivan, D. J. (2015). Antimalarial iron chelator FBS0701 blocks transmission by *Plasmodium falciparum* gametocyte activation inhibition. *Antimicrobial Agents and Chemotherapy*, *59*(3), 1418–1426.

Figueiredo, J. N., Räz, B., & Séquin, U. (1998). Novel quinone methides from *Salacia kraussii* with *in vitro* antimalarial activity. *Journal of Natural Products*, *61*(6), 718–723.

Filarsky, M., Fraschka, S. A., Niederwieser, I., Brancucci, N. M. B., Carrington, E., Carrió, E., Moes, S., Jenoe, P., Bártfai, R., & Voss, T. S. (2018). GDV1 induces

sexual commitment of malaria parasites by antagonizing HP1-dependent gene silencing. *Science*, *359*(6381), 1259 – 1263.

Flannery, E. L., Chatterjee, A. K., & Winzeler, E. A. (2013). Antimalarial drug discovery—approaches and progress towards new medicines. *Nature Reviews Microbiology*, *11*(12), 849–862.

Fleck, S. L., Pudney, M., & Sinden, R. E. (1996). The effect of atovaquone (566C80) on the maturation and viability of *Plasmodium falciparum* gametocytes *in vitro*. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, *90*(3), 309–312.

Fletcher, J. C., & Thummel, C. S. (1995). The ecdysone-inducible broad-complex and E74 early genes interact to regulate target gene transcription and *Drosophila* metamorphosis. *Genetics*, *141*(3), 1025–1035.

Fletcher, S., & Avery, V. M. (2014). A novel approach for the discovery of chemically diverse anti-malarial compounds targeting the *Plasmodium falciparum* Coenzyme A synthesis pathway. *Malaria Journal*, *13*(1), 343.

Florens, L., Washburn, M. P., Raine, J. D., Anthony, R. M., Grainger, M., Haynes, J. D., Moch, J. K., Muster, N., Sacci, J. B., Tabb, D. L., Witney, A. A., Wolters, D., Wu, Y., Gardner, M. J., Holder, A. A., Sinden, R. E., Yates, J. R., & Carucci, D. J. (2002). A proteomic view of the *Plasmodium falciparum* life cycle. *Nature*, *419*(6906), 520–526.

Fontenille, D., & Simard, F. (2004). Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations. *Comparative Immunology, Microbiology and Infectious Diseases*, *27*(5), 357–375.

Forkuo, A. D., Ansah, C., Mensah, K. B., Annan, K., Gyan, B., Theron, A., Mancama, D., & Wright, C. W. (2017). *In vitro* anti-malarial interaction and gametocytocidal activity of cryptolepine. *Malaria Journal*, *16*(1), 496.

Fouet, C., Atkinson, P., & Kamdem, C. (2018). Human interventions: Driving forces of mosquito evolution. *Trends in Parasitology*, *34*(2), 127–139.

Foy, B. D., Kobylinski, K. C., Silva, I. M. da, Rasgon, J. L., & Sylla, M. (2011). Endectocides for malaria control. *Trends in Parasitology*, *27*(10), 423–428.

Fraiture, M., Baxter, R. H. G., Steinert, S., Chelliah, Y., Frolet, C., Quispe-Tintaya, W., Hoffmann, J. A., Blandin, S. A., & Levashina, E. A. (2009). Two mosquito LRR proteins function as complement control factors in the TEP1-mediated killing of *Plasmodium*. *Cell Host & Microbe*, *5*(3), 273–284.

Francia, M. E., & Striepen, B. (2014). Cell division in apicomplexan parasites. *Nature Reviews Microbiology*, *12*(2), 125–136.

Francois, G., Timperman, G., Eling, W., Assi, L. A., Holenz, J., & Bringmann, G. (1997). Naphthylisoquinoline alkaloids against malaria: evaluation of the curative potentials of dioncophylline C and dioncopeltine A against *Plasmodium berghei* in vivo. *Antimicrobial Agents and Chemotherapy*, *41*(11), 2533–2539.

François, G, Timperman, G., Holenz, J., Assi, L. A., Geuder, T., Maes, L., Dubois, J., Hanocq, M., & Bringmannj, G. (1996). Naphthylisoquinoline alkaloids exhibit strong growth-inhibiting activities against *Plasmodium falciparum* and *P. berghei in vitro*—Structure—activity relationships of dioncophylline C. *Annals of Tropical Medicine & Parasitology*, *90*(2), 115–123.

François, Guido, Passreiter, C. M., Woerdenbag, H. J., & Van Looveren, M. (1996). Antiplasmodial activities and cytotoxic effects of aqueous extracts and sesquiterpene lactones from Neurolaena lobata. *Planta Medica*, *62*(02), 126–129.

Frédérich, M., De Pauw, M.-C., Prosperi, C., Tits, M., Brandt, V., Penelle, J., Hayette, M.-P., DeMol, P., & Angenot, L. (2001). Strychnogucines A and B, two new antiplasmodial bisindole alkaloids from *Strychnos icaja*. *Journal of Natural Products*, *64*(1), 12–16.

Frederich, M., Tits, M., & Angenot, L. (2008). Potential antimalarial activity of indole alkaloids. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, *102*(1), 11–19.

Frédérich, M., Tits, M., Goffin, E., Philippe, G., Grellier, P., De Mol, P., Hayette, M.-P., & Angenot, L. (2004). *In vitro* and *in vivo* antimalarial properties of

isostrychnopentamine, an indolomonoterpenic alkaloid from *Strychnos usambarensis*. *Planta Med*, *70*(06), 520–525.

Frosch, A. E. P., Laufer, M. K., Mathanga, D. P., Takala-Harrison, S., Skarbinski, J., Claassen, C. W., Dzinjalamala, F. K., & Plowe, C. V. (2014). Return of widespread chloroquine-sensitive *Plasmodium falciparum* to Malawi. *The Journal of Infectious Diseases*, *210*(7), 1110–1114.

Fuchs, S., Behrends, V., Bundy, J. G., Crisanti, A., & Nolan, T. (2014). Phenylalanine metabolism regulates reproduction and parasite melanization in the malaria mosquito. *PLOS ONE*, *9*(1), e84865.

Furuya, T., Mu, J., Hayton, K., Liu, A., Duan, J., Nkrumah, L., Joy, D. A., Fidock, D. A., Fujioka, H., Vaidya, A. B., Wellems, T. E., & Su, X. (2005). Disruption of a *Plasmodium falciparum* gene linked to male sexual development causes early arrest in gametocytogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(46), 16813–16818.

Futamura, Y., Muroi, M., & Osada, H. (2013). Target identification of small molecules based on chemical biology approaches. *Molecular BioSystems*, *9*(5), 897–914.

Gachimbi, L. N. (1990). Land degradation and its control in Kibwezi area, Kenya. University of Nairobi.

Gachimbi, L. N. (2002). Technical report on soil survey and sampling: Embu-Mbeere Districts, Kenya. *LUCID Project ILRI*, *9*.

Gadalla, A. A. H., Schneider, P., Churcher, T. S., Nassir, E., Abdel-muhsin, M. A., Ranford-cartwright, L. C., Reece, S. E., & Babiker, A. (2016). Associations between season and gametocyte dynamics in chronic *Plasmodium falciparum* infections. *PLoS One, 11(11)*, e0166699.

Gamo, F.-J., Sanz, L. M., Vidal, J., de Cozar, C., Alvarez, E., Lavandera, J.-L., Vanderwall, D. E., Green, D. V. S., Kumar, V., Hasan, S., Brown, J. R., Peishoff, C. E., Cardon, L. R., & Garcia-Bustos, J. F. (2010). Thousands of chemical starting points for antimalarial lead identification. *Nature*, *465*(7296), 305–310.

Ganesan, S., Chaurasiya, N. D., Sahu, R., Walker, L. A., & Tekwani, B. L. (2012).

Understanding the mechanisms for metabolism-linked hemolytic toxicity of primaquine against glucose 6-phosphate dehydrogenase deficient human erythrocytes: evaluation of eryptotic pathway. *Toxicology*, *294*(1), 54–60.

Garbi, M. I., Osman, E. E., Dahab, M. M., Koko, W. S., Kabbashi, A. S., Elegami, A. A., & Hamed, S. Y. (2014). Antigiardial, amoebicidal and cytotoxic activity of the plant *Prosopis juliflora* leave extracts. *Merit Research Journal of Biochemistry and Bioinformatics*, *2*(2), 1–7.

Gardiner, D. L., Dixon, M. W. A., Spielmann, T., Skinner-Adams, T. S., Hawthorne, P. L., Ortega, M. R., Kemp, D. J., & Trenholme, K. R. (2005). Implication of a *Plasmodium falciparum* gene in the switch between asexual reproduction and gametocytogenesis. *Molecular and Biochemical Parasitology*, *140*(2), 153–160.

Gbotosho, G. O., Sowunmi, A., Happi, C. T., & Okuboyejo, T. M. (2011). *Plasmodium falciparum* gametocyte carriage, sex ratios and asexual parasite rates in Nigerian children before and after a treatment protocol policy change instituting the use of artemisinin-based combination therapies. *Memórias do Instituto Oswaldo Cruz 106*, 685–690.

Gendrin, M, Turlure, F., Rodgers, F. H., Cohuet, A., Morlais, I., & Christophides, G. K. (2017). The peptidoglycan recognition proteins PGRPLA and PGRPLB regulate *Anopheles* immunity to bacteria and affect infection by *Plasmodium*. *Journal of Innate Immunity*, *9*(4), 333–342.

Gendrin, Mathilde, Rodgers, F. H., Yerbanga, R. S., Ouédraogo, J. B., Basáñez, M.-G., Cohuet, A., & Christophides, G. K. (2015). Antibiotics in ingested human blood affect the mosquito microbiota and capacity to transmit malaria. *Nature Communications*, *6*(1), 1–7.

George, D., Finn, R., Graham, K., & Sparagano, O. (2014). Present and future potential of plant-derived products to control arthropods of veterinary and medical significance. *Parasit Vectors*, *7*, 28.

Gessler, M. C., Msuya, D. E., Nkunya, M. H. H., Mwasumbi, L. B., Schär, A., Heinrich, M., & Tanner, M. (1995). Traditional healers in Tanzania: the treatment of malaria

with plant remedies. *Journal of Ethnopharmacology*, *48*(3), 131–144.

Gessler, M. C., Nkunya, M. H. H., Mwasumbi, L. B., Heinrich, M., & Tanner, M. (1994). Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropica*, *56*(1), 65–77.

Ghosh, A. K., Coppens, I., Gårdsvoll, H., Ploug, M., & Jacobs-lorena, M. (2011). *Plasmodium* ookinetes coopt mammalian plasminogen to invade the mosquito midgut. *Proceedings of the National Academy of Sciences*, 108(41), 17153 - 58.

Ghosh, A. K., Devenport, M., Jethwaney, D., Kalume, D. E., Pandey, A., Anderson, V. E., Sultan, A. a., Kumar, N., & Jacobs-Lorena, M. (2009). Malaria parasite invasion of the mosquito salivary gland requires interaction between the *Plasmodium* TRAP and the *Anopheles* saglin proteins. *PLoS Pathogens*, *5*(1), e1000265

Ghosh, A. K., Ribolla, P. E. M., & Jacobs-Lorena, M. (2001). Targeting *Plasmodium* ligands on mosquito salivary glands and midgut with a phage display peptide library. *Proceedings of the National Academy of Sciences*, *98*(23), 13278 – 13281.

Gianotti, R. L., Bomblies, A., Dafalla, M., Issa-Arzika, I., Duchemin, J.-B., & Eltahir, E. A. B. (2008). Efficacy of local neem extracts for sustainable malaria vector control in an African village. *Malaria Journal*, *7*(1), 138.

Ginsburg, H., & Deharo, E. (2011). A call for using natural compounds in the development of new antimalarial treatments – an introduction. *Malaria Journal*, *10*(1), S1.

Gissot, M., Ting, L.-M., Daly, T. M., Bergman, L. W., Sinnis, P., & Kim, K. (2008). High mobility group protein HMGB2 is a critical regulator of *Plasmodium* oocyst development. *The Journal of Biological Chemistry*, *283*(25), 17030–17038.

Gonçalves, B. P., Kapulu, M. C., Sawa, P., Guelbéogo, W. M., Tiono, A. B., Grignard, L., Stone, W., Hellewell, J., Lanke, K., Bastiaens, G. J. H., Bradley, J., Nébié, I., Ngoi, J. M., Oriango, R., Mkabili, D., Nyaurah, M., Midega, J., Wirth, D. F., Marsh, K., … Bousema, T. (2017). Examining the human infectious reservoir for *Plasmodium falciparum* malaria in areas of differing transmission intensity. *Nature Communications*, *8*(1), 1133.

Gonçalves, B. P., Tiono, A. B., Ouédraogo, A., Guelbéogo, W. M., Bradley, J., Nebie, I., Siaka, D., Lanke, K., Eziefula, A. C., Diarra, A., Pett, H., Bougouma, E. C., Sirima, S. B., Drakeley, C., & Bousema, T. (2016). Single low dose primaquine to reduce gametocyte carriage and *Plasmodium falciparum* transmission after artemether-lumefantrine in children with asymptomatic infection: a randomised, double-blind, placebo-controlled trial. *BMC Medicine*, *14*(1), 40.

González-Lázaro, M., Dinglasan, R. R., Hernández-Hernández, F. de la C., Rodríguez, M. H., Laclaustra, M., Jacobs-Lorena, M., & Flores-Romo, L. (2009). *Anopheles gambiae* Croquemort SCRBQ2, expression profile in the mosquito and its potential interaction with the malaria parasite *Plasmodium berghei*. *Insect Biochemistry and Molecular Biology*, *39*(5–6), 395–402.

Goulielmaki, E., & Loukeris, T. G. (2014). Functional characterization of *Anopheles* matrix metalloprotease 1 reveals its agonistic role during sporogonic development of malaria. *Infection and Immunity*, *82*(11), 4865–4877.

Green, J. L., Wu, Y., Encheva, V., Lasonder, E., Prommaban, A., Kunzelmann, S., Christodoulou, E., Grainger, M., Truongvan, N., Bothe, S., Sharma, V., Song, W., Pinzuti, I., Uthaipibull, C., Srichairatanakool, S., Birault, V., Langsley, G., Schindelin, H., Stieglitz, B., … Holder, A. A. (2020). Ubiquitin activation is essential for schizont maturation in *Plasmodium falciparum* blood-stage development. *PLoS Pathogens*, *16*(6), e1008640.

Grignard, L., Gonçalves, B. P., Early, A. M., Daniels, R. F., Tiono, A. B., Guelbéogo, W. M., Ouédraogo, A., van Veen, E. M., Lanke, K., Diarra, A., Nebie, I., Sirima, S. B., Targett, G. A., Volkman, S. K., Neafsey, D. E., Wirth, D. F., Bousema, T., & Drakeley, C. (2018). Transmission of molecularly undetectable circulating parasite clones leads to high infection complexity in mosquitoes post feeding. *International Journal for Parasitology*, *48*(8), 671–677.

Guantai, E., & Chibale, K. (2011). How can natural products serve as a viable source of lead compounds for the development of new / novel anti-malarials ? *Malaria Journal*, *10*(Suppl 1), 1–8.

Guelbéogo, W. M., Gonçalves, B. P., Grignard, L., Bradley, J., Serme, S. S., Hellewell, J., Lanke, K., Zongo, S., Sepúlveda, N., Soulama, I., Wangrawa, D. W., Yakob, L., Sagnon, N., Bousema, T., & Drakeley, C. (2018). Variation in natural exposure to anopheles mosquitoes and its effects on malaria transmission. *ELife*, *7*, e32625.

Guerreiro, A., Deligianni, E., Santos, J. M., Silva, P. A. G. C., Louis, C., Pain, A., Janse, C. J., Franke-Fayard, B., Carret, C. K., & Siden-Kiamos, I. (2014). Genome-wide RIP-Chip analysis of translational repressor-bound mRNAs in the *Plasmodium* gametocyte. *Genome Biology*, *15*(11), 1–16.

Guiguemde, W. A., Shelat, A. A., Bouck, D., Duffy, S., Crowther, G. J., Davis, P. H., Smithson, D. C., Connelly, M., Clark, J., & Zhu, F. (2010). Chemical genetics of *Plasmodium falciparum*. *Nature*, *465*(7296), 311–315.

Gulati, S., Ekland, E. H., Kelly, V., Marti, M., Di, G., Fidock, D. A., Gulati, S., Ekland, E. H., Ruggles, K. V, Chan, R. B., Jayabalasingham, B., Zhou, B., Mantel, P., Lee, M. C. S., Spottiswoode, N., Coburn-flynn, O., Hjelmqvist, D., Worgall, T. S., Marti, M., … Fidock, D. A. (2015). Profiling the essential nature of lipid metabolism in asexual blood and gametocyte stages of *Plasmodium falciparum*. *Cell Host and Microbe*, *18*(3), 371–381.

Gupta, L., Noh, J. Y., Jo, Y. H., Oh, S. H., Kumar, S., Noh, M. Y., Lee, Y. S., Cha, S.-J., Seo, S. J., & Kim, I. (2010). Apolipophorin-III mediates antiplasmodial epithelial responses in *Anopheles gambiae* (G3) mosquitoes. *PloS One*, *5*(11), e15410.

Guttery, D. S., Pittman, J. K., Frénal, K., Poulin, B., McFarlane, L. R., Slavic, K., Wheatley, S. P., Soldati-Favre, D., Krishna, S., Tewari, R., & Staines, H. M. (2013). The *Plasmodium berghei* Ca2+/H+ Exchanger, PbCAX, is essential for tolerance to environmental Ca2+ during sexual development. *PLOS Pathogens*, *9*(2), e1003191.

Guttery, D. S., Poulin, B., Ferguson, D. J. P., Szöőr, B., Wickstead, B., Carroll, P. L., Ramakrishnan, C., Brady, D., Patzewitz, E.-M., Straschil, U., Solyakov, L., Green, J. L., Sinden, R. E., Tobin, A. B., Holder, A. A., & Tewari, R. (2012). A unique protein phosphatase with kelch-like domains (PPKL) in *Plasmodium* modulates

ookinete differentiation, motility and invasion. *PLOS Pathogens*, *8*(9), e1002948.

Guttery, D. S., Poulin, B., Ramaprasad, A., Wall, R. J., Ferguson, D. J. P., Brady, D., Patzewitz, E. M., Whipple, S., Straschil, U., Wright, M. H., Mohamed, A. M. a H., Radhakrishnan, A., Arold, S. T., Tate, E. W., Holder, A. a., Wickstead, B., Pain, A., & Tewari, R. (2014). Genome-wide functional analysis of *Plasmodium* protein phosphatases reveals key regulators of parasite development and differentiation. In *Cell Host and Microbe 16 (1)*, 128–140.

Hackney, J. F., Zolali-Meybodi, O., & Cherbas, P. (2012). Tissue damage disrupts developmental progression and ecdysteroid biosynthesis in *Drosophila*. *PLOS ONE*, *7*(11), e49105.

Han, Y. S., Thompson, J., Kafatos, F. C., & Barillas-Mury, C. (2000). Molecular interactions between *Anopheles stephensi* midgut cells and *Plasmodium berghei*: the time bomb theory of ookinete invasion of mosquitoes. *The EMBO Journal*, *19*(22), 6030–6040.

Harmatha, J., & Dinan, L. (2003). Biological activities of lignans and stilbenoids associated with plant-insect chemical interactions. *Phytochemistry Reviews*, *2*(3), 321–330.

Hart, R. J., Cornillot, E., Abraham, A., Molina, E., Nation, C. S., Mamoun, C. Ben, & Aly, A. S. I. (2016). Genetic characterization of *Plasmodium* putative pantothenate kinase genes reveals their essential role in malaria parasite transmission to the mosquito. *Scientific Reports*, *6*, 33518.

Hata, Y., Zimmermann, S., Quitschau, M., Kaiser, M., Hamburger, M., & Adams, M. (2011). Antiplasmodial and antitrypanosomal activity of pyrethrins and pyrethroids. *Journal of Agricultural and Food Chemistry*, *59*(17), 9172–9176.

He, G., Sun, Y., & Li, F. (2012). RNA interference of two acetylcholinesterase genes in *Plutella xylostella* reveals their different functions. *Archives of Insect Biochemistry and Physiology*, *79*(2), 75–86.

He, Q., Wang, W., & Zhu, L. (2018). Larvicidal activity of *Zanthoxylum acanthopodium* essential oil against the malaria mosquitoes, *Anopheles anthropophagus* and

Anopheles sinensis. *Malaria Journal*, *17*(1), 194.

Held, J., Jeyaraj, S., & Kreidenweiss, A. (2015). Antimalarial compounds in Phase II clinical development. *Expert Opinion on Investigational Drugs*, *24*(3), 363–382.

Hematpoor, A., Liew, S. Y., Chong, W. L., Azirun, M. S., Lee, V. S., & Awang, K. (2016). Inhibition and larvicidal activity of phenylpropanoids from *Piper sarmentosum* on acetylcholinesterase against mosquito vectors and their binding mode of interaction. *PLOS ONE*, *11*(5), e0155265.

Hempelmann, E., & Krafts, K. (2013). Bad air, amulets and mosquitoes: 2,000 years of changing perspectives on malaria. *Malaria Journal*, *12*(1), 232.

Henciya, S., Seturaman, P., James, A. R., Tsai, Y.-H., Nikam, R., Wu, Y.-C., Dahms, H.-U., & Chang, F. R. (2017). Biopharmaceutical potentials of *Prosopis* spp.(Mimosaceae, Leguminosa). *Journal of Food and Drug Analysis*, *25*(1), 187–196.

Hill, W. G., & Babiker, H. A. (1995). Estimation of numbers of malaria clones in blood samples. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *262*(1365), 249–257.

Hirasawa, Y., Arai, H., Zaima, K., Oktarina, R., Rahman, A., Ekasari, W., Widyawaruyanti, A., Indrayanto, G., Zaini, N. C., & Morita, H. (2009). Alstiphyllanines A−D, indole alkaloids from *Alstonia macrophylla*. *Journal of Natural Products*, *72*(2), 304–307.

Hirunpanich, V., Sethabouppha, B., & Sato, H. (2007). Inhibitory effects of saturated and polyunsaturated fatty acids on the cytochrome P450 3A activity in rat liver microsomes. *Biological and Pharmaceutical Bulletin*, *30*(8), 1586–1588.

Hoepfner, D., Mcnamara, C. W., Lim, C. S., Studer, C., Riedl, R., Aust, T., Mccormack, S. L., Plouffe, D. M., Meister, S., Schuierer, S., Plikat, U., Hartmann, N., Staedtler, F., Cotesta, S., Schmitt, E. K., Petersen, F., Supek, F., Glynne, R. J., Tallarico, J. A., … Movva, N. R. (2012). Selective and specific inhibition of the *Plasmodium falciparum* lysyl-tRNA synthetase by the fungal secondary metabolite cladosporin. *Cell Host and Microbe*, *11*(6), 654–663.

Hopp, C. S., Balaban, A. E., Bushell, E. S. C., Billker, O., Rayner, J. C., & Sinnis, P. (2016). Palmitoyl transferases have critical roles in the development of mosquito and liver stages of *Plasmodium*. *Cellular Microbiology*, *18*(11), 1625–1641.

Hsu, Y.-C., Chiu, Y.-T., Cheng, C.-C., Wu, C.-F., Lin, Y.-L., & Huang, Y.-T. (2007). Antifibrotic effects of tetrandrine on hepatic stellate cells and rats with liver fibrosis. *Journal of Gastroenterology and Hepatology*, *22*(1), 99–111.

Huang, F., Takala-Harrison, S., Jacob, C. G., Liu, H., Sun, X., Yang, H., Nyunt, M. M., Adams, M., Zhou, S., & Xia, Z. (2015). A single mutation in K13 predominates in southern China and is associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment. *The Journal of Infectious Diseases*, *212*(10), 1629–1635.

Hughes, R. E., Elliott, R. J. R., Dawson, J. C., & Carragher, N. O. (2021). High-content phenotypic and pathway profiling to advance drug discovery in diseases of unmet need. *Cell Chemical Biology*, *28*(3), 338–355.

Hui, X., Yang, L., He, G., Yang, Q., Han, Z., & Li, F. (2011). RNA interference of ace1 and ace2 in *Chilo suppressalis* reveals their different contributions to motor ability and larval growth. *Insect Molecular Biology*, *20*(4), 507–518.

Imbahale, S. S., & Mukabana, W. R. (2015). Efficacy of neem chippings for mosquito larval control under field conditions. *BMC Ecology*, *15*(1), 1.

Innocent, E., Gikonyo, N. K., & Nkunya, M. H. (2008). Repellency property of long chain aliphatic methyl ketones against *Anopheles gambiae* ss. *Tanzania Journal of Health Research*, *10*(1), 50–54.

Inoue, J., Lopes, D., do Rosário, V., Machado, M., Hristov, A. D., Lima, G. F., Costa-Nascimento, M. J., Segurado, A. C., & Di Santi, S. M. (2014). Analysis of polymorphisms in *Plasmodium falciparum* genes related to drug resistance: a survey over four decades under different treatment policies in Brazil. *Malaria Journal*, *13*, 372.

Ishiyama, A., Iwatsuki, M., Namatame, M., Nishihara-Tsukashima, A., Sunazuka, T., Takahashi, Y., Ōmura, S., & Otoguro, K. (2011). Borrelidin, a potent antimalarial:

stage-specific inhibition profile of synchronized cultures of *Plasmodium falciparum*. *The Journal of Antibiotics*, *64*(5), 381–384.

Ito, J., Ghosh, A., Moreira, L. A., Wimmer, E. A., & Jacobs-Lorena, M. (2002). Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature*, *417*(6887), 452–455.

Janse, C. J., van der Klooster, P. F. J., van der Kaay, H. J., van der Ploeg, M., & Overdulve, J. P. (1986). DNA synthesis in *Plasmodium berghei* during asexual and sexual development. *Molecular and Biochemical Parasitology*, *20*(2), 173–182.

Jaramillo-Gutierrez, G., Molina-Cruz, A., Kumar, S., & Barillas-Mury, C. (2010). The *Anopheles gambiae* oxidation resistance 1 (OXR1) gene regulates expression of enzymes that detoxify reactive oxygen species. *PLoS ONE*, *5*(6), e11168.

Jean-Jacques, L., Jean-Paul, D., & Jean, G. (2005). Developmental changes and acetylcholinesterase activity in the metamorphosing brain of *Tenebrio molitor*: Correlation to ecdysteroid titers. *Archives of Insect Biochemistry and Physiology*, *25*(3), 207–226.

Jennison, C., Lucantoni, L., O'Neill, M. T., McConville, R., Erickson, S. M., Cowman, A. F., Sleebs, B. E., Avery, V. M., & Boddey, J. A. (2019). Inhibition of plasmepsin V activity blocks *Plasmodium falciparum* gametocytogenesis and transmission to mosquitoes. *Cell Reports*, *29*(12), 3796–3806.

Jenson, L. J., Paulson, S. L., & Bloomquist, J. R. (2012). Induction and inhibition of an apparent neuronal phenotype in *Spodoptera frugiperda* insect cells (Sf21) by chemical agents. *Invertebrate Neuroscience*, *12*(2), 119–127.

Joice, R., Nilsson, S. K., Montgomery, J., Dankwa, S., Egan, E., Morahan, B., Seydel, K. B., Bertuccini, L., Alano, P., Williamson, K. C., Duraisingh, M. T., Taylor, T. E., Milner, D. A., & Marti, M. (2014). *Plasmodium falciparum* transmission stages accumulate in the human bone marrow. *Science Translational Medicine*, *6*(244), 244re5-244re5.

Jones, I. W., Denholm, A. A., Ley, S. V, Lovell, H., Wood, A., & Sinden, R. E. (1994). Sexual development of malaria parasites is inhibited in vitro by the Neem extract

Azadirachtin, and its semi-synthetic analogues. *FEMS Microbiology Letters*, *120*(3), 267–273.

Jones, T. R., & Hoffman, S. L. (1994). Malaria vaccine development. *Clinical Microbiology Reviews*, *7*(3), 303–310.

Kadota, K., Ishino, T., Matsuyama, T., Chinzei, Y., & Yuda, M. (2004). Essential role of membrane-attack protein in malarial transmission to mosquito host. *Proceedings of the National Academy of Sciences*, *101*(46), 16310–16315.

Kafsack, B. F. C., Rovira-Graells, N., Clark, T. G., Bancells, C., Crowley, V. M., Campino, S. G., Williams, A. E., Drought, L. G., Kwiatkowski, D. P., Baker, D. A., Cortés, A., & Llinás, M. (2014). A transcriptional switch underlies commitment to sexual development in malaria parasites. *Nature*, *507*, 248.

Kalauni, S. K., Awale, S., Tezuka, Y., Banskota, A. H., Linn, T. Z., Asih, P. B. S., Syafruddin, D., & Kadota, S. (2006). Antimalarial activity of cassane- and norcassane-type diterpenes from *Caesalpinia crista* and their structure–activity relationship. *Biological and Pharmaceutical Bulletin*, *29*(5), 1050–1052.

Kamaraj, C., Rahuman, A. A., & Roopan, S. M. (2014). Bioassay-guided isolation and characterization of active antiplasmodial compounds from *Murraya koenigii* extracts against *Plasmodium falciparum* and *Plasmodium berghei*. *Parasitology Research*, *113*(5), 1657–1672.

Kamau, E., Campino, S., Amenga-Etego, L., Drury, E., Ishengoma, D., Johnson, K., Mumba, D., Kekre, M., Yavo, W., Mead, D., Bouyou-Akotet, M., Apinjoh, T., Golassa, L., Randrianarivelojosia, M., Andagalu, B., Maiga-Ascofare, O., Amambua-Ngwa, A., Tindana, P., Ghansah, A., … Djimde, A. A. (2015). K13-Propeller polymorphisms in *Plasmodium falciparum* parasites from sub-Saharan Africa. *The Journal of Infectious Diseases*, *211*(8), 1352–1355.

Kamsuk, K., Choochote, W., Chaithong, U., Jitpakdi, A., Tippawangkosol, P., Riyong, D., & Pitasawat, B. (2007). Effectiveness of *Zanthoxylum piperitum*-derived essential oil as an alternative repellent under laboratory and field applications. *Parasitology Research*, *100*(2), 339.

Kapulu, M. C., Da, D. F., Miura, K., Li, Y., Blagborough, A. M., Churcher, T. S., Nikolaeva, D., Williams, A. R., Goodman, A. L., Sangare, I., Turner, A. V, Cottingham, M. G., Nicosia, A., Straschil, U., Tsuboi, T., Gilbert, S. C., Long, C. A., Sinden, R. E., Draper, S. J., … Biswas, S. (2015). Comparative assessment of transmission-blocking vaccine candidates against *Plasmodium falciparum*. *Scientific Reports*, *5*, 11193.

Kato, A., Moriyasu, M., Ichimaru, M., Nishiyama, Y., Juma, F. D., Nganga, J. N., Mathenge, S. G., & Ogeto, J. O. (1996). Isolation of alkaloidal constituents of *Zanthoxylum usambarense* and *Zanthoxylum chalybeum* using ion-pair HPLC. *Journal of Natural Products*, *59*(3), 316–318.

Kaur, K., Jain, M., Kaur, T., & Jain, R. (2009). Antimalarials from nature. *Bioorganic & Medicinal Chemistry*, *17*(9), 3229–3256.

Ke, H., Lewis, I. A., Morrisey, J. M., McLean, K. J., Ganesan, S. M., Painter, H. J., Mather, M. W., Jacobs-Lorena, M., Llinás, M., & Vaidya, A. B. (2015). Genetic investigation of tricarboxylic acid metabolism during the *Plasmodium falciparum* life cycle. *Cell Reports*, *11*(1), 164–174.

Kent, K. S., Consoulas, C., Duncan, K., Johnston, R. M., Luedeman, R., & Levine, R. B. (1995). Remodelling of neuromuscular systems during insect metamorphosis. *American Zoologist*, *35*(6), 578–584.

Kent, R. S., Modrzynska, K. K., Cameron, R., Philip, N., Billker, O., & Waters, A. P. (2018). Inducible developmental reprogramming redefines commitment to sexual development in the malaria parasite *Plasmodium berghei*. *Nature Microbiology*, *3*(11), 1206–1213.

Kern, S., Agarwal, S., Huber, K., Gehring, A. P., Ströedke, B., Wirth, C. C., Brügl, T., Abodo, L. O., Dandekar, T., & Doerig, C. (2014). Inhibition of the SR protein-phosphorylating CLK kinases of *Plasmodium falciparum* impairs blood stage replication and malaria transmission. *PloS One*, *9*(9), e105732.

Keter, L. K., & Mutiso, P. C. (2012). Ethnobotanical studies of medicinal plants used by traditional health practitioners in the management of diabetes in Lower Eastern

Province, Kenya. *Journal of Ethnopharmacology*, *139*(1), 74–80.

Khan, S. M., Franke-fayard, B., Mair, G. R., Lasonder, E., Janse, C. J., Mann, M., & Waters, A. P. (2005). Proteome analysis of separated male and female gametocytes reveals novel sex-specific *Plasmodium* biology. *121*, 675–687.

Khater, E. I., Sinden, R. E., & Dessens, J. T. (2004). A malaria membrane skeletal protein is essential for normal morphogenesis, motility, and infectivity of sporozoites. *The Journal of Cell Biology*, *167*(3), 425–432.

Kiarie, W. C., Wangai, L., Agola, E., Kimani, F. T., & Hungu, C. (2015). Chloroquine sensitivity: diminished prevalence of chloroquine-resistant gene marker pfcrt-76 13 years after cessation of chloroquine use in Msambweni, Kenya. *Malaria Journal*, *14*(1), 328.

Kiboi, D., Irungu, B., Orwa, J., Kamau, L., Ochola-Oyier, L. I., Ngángá, J., & Nzila, A. (2014). Piperaquine and Lumefantrine resistance in *Plasmodium berghei* ANKA associated with increased expression of Ca(2+)/H(+) antiporter and glutathione associated enzymes. *Experimental Parasitology*, *147*, 23–32.

Kilama, M., Smith, D. L., Hutchinson, R., Kigozi, R., Yeka, A., Lavoy, G., Kamya, M. R., Staedke, S. G., Donnelly, M. J., Drakeley, C., Greenhouse, B., Dorsey, G., & Lindsay, S. W. (2014). Estimating the annual entomological inoculation rate for *Plasmodium falciparum* transmitted by *Anopheles gambiae* s.l. using three sampling methods in three sites in Uganda. *Malaria Journal*, *13*(1), 111.

Killeen, G. F. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malaria Journal*, *13*(1), 330.

Killeen, G. F., Fillinger, U., Kiche, I., Gouagna, L. C., & Knols, B. G. J. (2002). Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? *The Lancet Infectious Diseases*, *2*(10), 618–627.

Killeen, G. F., Fillinger, U., & Knols, B. G. J. (2002). Advantages of larval control for African malaria vectors: Low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malaria Journal*, *1*, 8.

Killeen, G. F., Marshall, J. M., Kiware, S. S., South, A. B., Tusting, L. S., Chaki, P. P.,

& Govella, N. J. (2017). Measuring, manipulating and exploiting behaviours of adult mosquitoes to optimise malaria vector control impact. *BMJ Global Health*, *2*(2), e000212.

Kim, S.-I., & Ahn, Y.-J. (2017). Larvicidal activity of lignans and alkaloid identified in *Zanthoxylum piperitum* bark toward insecticide-susceptible and wild *Culex pipiens* pallens and *Aedes aegypti*. *Parasites & Vectors*, *10*(1), 221.

Kimps, N. W., Bissinger, B. W., Apperson, C. S., Sonenshine, D. E., & Roe, R. M. (2011). First report of the repellency of 2-tridecanone against ticks. *Medical and Veterinary Entomology*, *25*(2), 202–208.

Kobylinski, K C, Sylla, M., Chapman, P. L., Sarr, M. D., & Foy, B. D. (2011). Ivermectin mass drug administration to humans disrupts malaria parasite transmission in Senegalese villages. *Am J Trop Med Hyg*, *85*(1), 3–5.

Kobylinski, Kevin C, Deus, K. M., Butters, M. P., Hongyu, T., Gray, M., da Silva, I. M., Sylla, M., & Foy, B. D. (2010). The effect of oral anthelmintics on the survivorship and re-feeding frequency of anthropophilic mosquito disease vectors. *Acta Tropica*, *116*(2), 119–126.

Kobylinski, Kevin C, Escobedo-Vargas, K. S., López-Sifuentes, V. M., Durand, S., Smith, E. S., Baldeviano, G. C., Gerbasi, R. V, Ballard, S.-B., Stoops, C. A., & Vásquez, G. M. (2017). Ivermectin susceptibility, sporontocidal effect, and inhibition of time to re-feed in the Amazonian malaria vector *Anopheles darlingi*. *Malaria Journal*, *16*(1), 474.

Kobylinski, Kevin C, Foy, B. D., & Richardson, J. H. (2012). Ivermectin inhibits the sporogony of *Plasmodium falciparum* in *Anopheles gambiae*. *Malaria Journal*, *11*(1), 381.

Kokate, S. D., Venkatachalam, S. R., & Hassarajani, S. A. (2001). *Zanthoxylum alatum* extract as mosquito larvicide. *Proceedings of National Academy of Sciences India Section B*, *71*(3/4), 229–232.

Kokwaro, J. O. (1993). Medicinal plants of east Africa. *Nairobi: Kenya Literature Bureau 401p. ISBN*, *9966*(44), 190.

Kooij, T. W. A., & Matuschewski, K. (2007). Triggers and tricks of *Plasmodium* sexual development. *Current Opinion in Microbiology*, *10*(6), 547–553.

Koyama, T., & Mirth, C. K. (2018). Unravelling the diversity of mechanisms through which nutrition regulates body size in insects. *Current Opinion in Insect Science*, *25*, 1–8.

Krane, B. D., Fagbule, M. O., Shamma, M., & Gözler, B. (1984). The benzophenanthridine alkaloids. *Journal of Natural Products*, *47*(1), 1–43.

Kubo, M., Yatsuzuka, W., Matsushima, S., Harada, K., Inoue, Y., Miyamoto, H., Matsumoto, M., & Fukuyama, Y. (2016). Antimalarial phenanthroindolizine alkaloids from Ficus septica. *Chemical and Pharmaceutical Bulletin*, *64*(7), 957–960.

Kuehn, A., & Pradel, G. (2010). The coming-out of malaria gametocytes. *Journal of Biomedicine and Biotechnology, 2010*, 976827.

Kuhn, S., Gill, M. J., & Kain, K. C. (2005). Emergence of atovaquone-proguanil resistance during treatment of *Plasmodium falciparum* malaria acquired by a non-immune north American traveller to west Africa. *The American Journal of Tropical Medicine and Hygiene*, *72*(4), 407–409.

Kumar, M., Gupta, G. P., & Rajam, M. V. (2009). Silencing of acetylcholinesterase gene of *Helicoverpa armigera* by siRNA affects larval growth and its life cycle. *Journal of Insect Physiology*, *55*(3), 273–278.

Kumar, S., Christophides, G. K., Cantera, R., Charles, B., Han, Y. S., Meister, S., Dimopoulos, G., Kafatos, F. C., & Barillas-mury, C. (2003). The role of reactive oxygen species on *Plasmodium* melanotic encapsulation in *Anopheles gambiae*. *Proceedings of the National Academy of Sciences, 100*(24), 14139-14144.

Kumar, S., Gupta, L., Han, Y. S., & Barillas-Mury, C. (2004). Inducible peroxidases mediate nitration of *Anopheles* midgut cells undergoing apoptosis in response to *Plasmodium* invasion. *Journal of Biological Chemistry*, *279*(51), 53475–53482.

Kumar, S., Molina-Cruz, A., Gupta, L., Rodrigues, J., & Barillas-Mury, C. (2010). A peroxidase/dual oxidase system modulates midgut epithelial immunity in *Anopheles*

gambiae. *Science*, *327*(5973), 1644 – 1648.

Kumnuan, R., Pattaradilokrat, S., Chumpolbanchorn, K., Pimnon, S., Narkpinit, S., Harnyuttanakorn, P., & Saiwichai, T. (2013). *In vivo* transmission blocking activities of artesunate on the avian malaria parasite *Plasmodium gallinaceum*. *Veterinary Parasitology*, *197*(3), 447–454.

Kunert, O., Swamy, R. C., Kaiser, M., Presser, A., Buzzi, S., Rao, A. V. N. A., & Schühly, W. (2008). Antiplasmodial and leishmanicidal activity of biflavonoids from Indian *Selaginella bryopteris*. *Phytochemistry Letters*, *1*(4), 171–174.

Kuo, P.-C., Damu, A. G., Lee, K.-H., & Wu, T.-S. (2004). Cytotoxic and antimalarial constituents from the roots of *Eurycoma longifolia*. *Bioorganic & Medicinal Chemistry*, *12*(3), 537–544.

Kupchan, S. M., Yokoyama, N., & Beal, J. L. (1960). Menispermaceae Alkaloids I: The alkaloids of *Cissampelos pareira* Linn. and the origin of *Radix Pareirae* Bravae. *Journal of the American Pharmaceutical Association (Scientific Ed.)*, *49*(11), 727–731.

Kweka, E. J., Munga, S., Mahande, A. M., Msangi, S., Mazigo, H. D., Adrias, A. Q., & Matias, J. R. (2012). Protective efficacy of menthol propylene glycol carbonate compared to N, N-diethyl-methylbenzamide against mosquito bites in Northern Tanzania. *Parasites & Vectors*, *5*(1), 189.

Lacroix, R., Mukabana, W. R., Gouagna, L. C., & Koella, J. C. (2005). Malaria infection increases attractiveness of humans to mosquitoes. *PLOS Biology*, *3*(9), e298.

Lal, K., Delves, M. J., Bromley, E., Wastling, J. M., Tomley, F. M., & Sinden, R. E. (2009). *Plasmodium* male development gene-1 (mdv-1) is important for female sexual development and identifies a polarised plasma membrane during zygote development. *International Journal for Parasitology*, *39*(7), 755–761.

Lasonder, E., Janse, C. J., van Gemert, G.-J., Mair, G. R., Vermunt, A. M. W., Douradinha, B. G., van Noort, V., Huynen, M. A., Luty, A. J. F., Kroeze, H., Khan, S. M., Sauerwein, R. W., Waters, A. P., Mann, M., & Stunnenberg, H. G. (2008). Proteomic profiling of *Plasmodium* sporozoite maturation identifies new proteins

essential for parasite development and infectivity. *PLOS Pathogens*, *4*(10), e1000195.

Lasonder, E., Rijpma, S. R., Schaijk, B. C. L. Van, Hoeijmakers, W. A. M., Kensche, P. R., Gresnigt, M. S., Italiaander, A., Vos, M. W., Woestenenk, R., Bousema, T., Mair, G. R., Khan, S. M., Janse, C. J., & Sauerwein, R. W. (2016). Integrated transcriptomic and proteomic analyses of *P. falciparum* gametocytes : molecular insight into sex-specific processes and translational repression. *Nucleic Acids Research*, *44*(13), 6087–6101.

Laufer, M. K., Takala-Harrison, S., Dzinjalamala, F. K., Stine, O. C., Taylor, T. E., & Plowe, C. V. (2010). Return of chloroquine-susceptible *falciparum* malaria in Malawi was a reexpansion of diverse susceptible parasites. *The Journal of Infectious Diseases*, *202*(5), 801–808.

Laurens, M. B. (2018). The promise of a malaria vaccine—Are we closer? *Annual Review of Microbiology*, *72*(1), 273–292.

Laurentino, E. C., Taylor, S., Mair, G. R., Lasonder, E., Bartfai, R., Stunnenberg, H. G., Kroeze, H., Ramesar, J., Franke-Fayard, B., Khan, S. M., Janse, C. J., & Waters, A. P. (2011). Experimentally controlled downregulation of the histone chaperone FACT in *Plasmodium berghei* reveals that it is critical to male gamete fertility. *Cellular Microbiology*, *13*(12), 1956–1974.

Lavazec, C., Boudin, C., Lacroix, R., Bonnet, S., Diop, A., Thiberge, S., Boisson, B., Tahar, R., & Bourgouin, C. (2007). Carboxypeptidases B of *Anopheles gambiae* as targets for a *Plasmodium falciparum* transmission-blocking vaccine. *Infection and Immunity*, *75*(4), 1635–1642.

Le Roch, K. G., Johnson, J. R., Florens, L., Zhou, Y., Santrosyan, A., Grainger, M., Yan, S. F., Williamson, K. C., Holder, A. A., Carucci, D. J., Yates 3rd, J. R., & Winzeler, E. A. (2004). Global analysis of transcript and protein levels across the *Plasmodium falciparum* life cycle. *Genome Research*, *14*(11), 2308–2318.

Lecona-Valera, A. N., Tao, D., Rodríguez, M. H., López, T., Dinglasan, R. R., & Rodríguez, M. C. (2016). An antibody against an *Anopheles albimanus* midgut

myosin reduces *Plasmodium berghei* oocyst development. *Parasites & Vectors*, *9*(1), 274.

Lee, J., & Bogyo, M. (2013). Target deconvolution techniques in modern phenotypic profiling. *Current Opinion in Chemical Biology*, *17*(1), 118–126.

Lee, S.-H., Oh, H.-W., Fang, Y., An, S.-B., Park, D.-S., Song, H.-H., Oh, S.-R., Kim, S.-Y., Kim, S., & Kim, N. (2015). Identification of plant compounds that disrupt the insect juvenile hormone receptor complex. *Proceedings of the National Academy of Sciences*, *112*(6), 1733–1738.

Lefevre, T., Ohm, J., Dabiré, K. R., Cohuet, A., Cator, L., Choisy, M., & Thomas, M. B. (2017). Transmission traits of malaria parasites within the mosquito : Genetic variation, phenotypic plasticity, and consequences for control. *Evolutionary Applications*, *11*(4), 456 - 469.

Leite, F. C., Mello, C. da S., Fialho, L. G., Marinho, C. F., Lima, A. L. de A., Barbosa Filho, J. M., Kubelka, C. F., & Piuvezam, M. R. (2016). *Cissampelos sympodialis* has anti-viral effect inhibiting dengue non-structural viral protein-1 and pro-inflammatory mediators. *Revista Brasileira de Farmacognosia*, *26*(4), 502–506.

Lelièvre, J., Almela, M. J., Lozano, S., Miguel, C., Franco, V., Leroy, D., & Herreros, E. (2012). Activity of clinically relevant antimalarial drugs on *Plasmodium falciparum* mature gametocytes in an ATP bioluminescence “transmission blocking” assay. *PLOS ONE*, *7*(4), e35019.

Lengai, G. M. W., Muthomi, J. W., & Mbega, E. R. (2019). Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Scientific African*, e00239.

Li, G., Peng, X., Guo, Y., Gong, S., Cao, S., & Qiu, F. (2021). Currently available strategies for target identification of bioactive natural products. *Frontiers in Chemistry*, 830.

Li, H., & Cooper, R. L. (2001). Effects of the ecdysoneless mutant on synaptic efficacy and structure at the neuromuscular junction in *Drosophila* larvae during normal and prolonged development. *Neuroscience*, *106*(1), 193–200.

Li, J. W.-H., & Vederas, J. C. (2009). Drug discovery and natural products: end of an era or an endless frontier? *Science*, *325*(5937), 161–165.

Li, M. W. M., Wang, J., Zhao, Y. O., & Fikrig, E. (2014). Innexin AGAP001476 is critical for mediating anti-*Plasmodium* responses in *Anopheles* mosquitoes. *The Journal of Biological Chemistry*, *289*(36), 24885–24897.

Likhitwitayawuid, K., Kaewamatawong, R., Ruangrungsi, N., & Krungkrai, J. (1998). Antimalarial naphthoquinones from *Nepenthes thorelii*. *Planta Medica*, *64*(03), 237–241.

Lim, Y.-P., Ma, C.-Y., Liu, C.-L., Lin, Y.-H., Hu, M.-L., Chen, J.-J., Hung, D.-Z., Hsieh, W.-T., & Huang, J.-D. (2012). Sesamin: a naturally occurring lignan inhibits CYP3A4 by antagonizing the pregnane X receptor activation. *Evidence-Based Complementary and Alternative Medicine*, *2012*(242810), 1–15.

Lima, H. G., Gomes, D. C., Santos, N. S., Dias, Ê. R., Botura, M. B., Batatinha, M. J. M., & Branco, A. (2017). *Prosopis juliflora* pods alkaloid-rich fraction: *In vitro* anthelmintic activity on goat gastrointestinal parasites and its cytotoxicity on vero cells. *Pharmacognosy Magazine*, *13*(Suppl 3), S684.

Liu, X. C., Liu, Q. Y., Zhou, L., Liu, Q. R., & Liu, Z. L. (2014). Chemical composition of *Zanthoxylum avicennae* essential oil and its larvicidal activity on Aedes albopictus Skuse. *Tropical Journal of Pharmaceutical Research*, *13*(3), 399–404.

Liu, Y., Tewari, R., Ning, J., Blagborough, A. M., Garbom, S., Pei, J., Grishin, N. V, Steele, R. E., Sinden, R. E., & Snell, W. J. (2008). The conserved plant sterility gene HAP2 functions after attachment of fusogenic membranes in *Chlamydomonas* and *Plasmodium* gametes. *Genes & Development*, *22*(8), 1051–1068.

Liu, Z., Lv, H., Li, H., Zhang, Y., Zhang, H., Su, F., Xu, S., Li, Y., Si, Y., & Yu, S. (2011). Interaction studies of an anticancer alkaloid,(+)-(13aS)-deoxytylophorinine, with calf thymus DNA and four repeated double-helical DNAs. *Chemotherapy*, *57*(4), 310–320.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, *25*(4), 402–408.

Lombardo, F., Ghani, Y., Kafatos, F. C., & Christophides, G. K. (2013). Comprehensive genetic dissection of the hemocyte immune response in the malaria mosquito *Anopheles gambiae*. *PLOS Pathogens*, *9*(1), e1003145.

Lopez-Rubio, J.-J., Mancio-Silva, L., & Scherf, A. (2009). Genome-wide analysis of heterochromatin associates clonally variant gene regulation with perinuclear repressive centers in malaria parasites. *Cell Host & Microbe*, *5*(2), 179–190.

Lu, Y., Park, Y., Gao, X., Zhang, X., Yao, J., Pang, Y.-P., Jiang, H., & Zhu, K. Y. (2012). Cholinergic and non-cholinergic functions of two acetylcholinesterase genes revealed by gene-silencing in *Tribolium castaneum*. *Scientific Reports*, *2*, 288.

Lucantoni, L, Giusti, F., Cristofaro, M., Pasqualini, L., Esposito, F., Lupetti, P., & Habluetzel, A. (2006). Effects of a neem extract on blood feeding, oviposition and oocyte ultrastructure in *Anopheles stephensi* Liston (Diptera: Culicidae). *Tissue and Cell*, *38*(6), 361–371.

Lucantoni, Leonardo, Yerbanga, R. S., Lupidi, G., Pasqualini, L., Esposito, F., & Habluetzel, A. (2010). Transmission blocking activity of a standardized neem (*Azadirachta indica*) seed extract on the rodent malaria parasite *Plasmodium berghei* in its vector *Anopheles stephensi*. *Malaria Journal*, *9*(1), 66.

Luckhart, S., Pakpour, N., & Giulivi, C. (2015). Host-pathogen interactions in malaria: cross-kingdom signaling and mitochondrial regulation. *Current Opinion in Immunology*, *36*, 73–79.

Lyimo, I. N., Kessy, S. T., Mbina, K. F., Daraja, A. A., & Mnyone, L. L. (2017). Ivermectin-treated cattle reduces blood digestion, egg production and survival of a free-living population of *Anopheles arabiensis* under semi-field condition in south-eastern Tanzania. *Malaria Journal*, *16*(1), 239.

Macdonald, G. (1957). The epidemiology and control of malaria. *The Epidemiology and Control of Malaria.*

Mahmoudi, N., de Julián-Ortiz, J.-V., Ciceron, L., Gálvez, J., Mazier, D., Danis, M., Derouin, F., & García-Domenech, R. (2006). Identification of new antimalarial

drugs by linear discriminant analysis and topological virtual screening. *Journal of Antimicrobial Chemotherapy*, *57*(3), 489–497.

Maia, M. F., & Moore, S. J. (2011). Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal*, *10*(Suppl 1), S11–S11.

Maioli, M. A., Lemos, D. E. C. V, Guelfi, M., Medeiros, H. C. D., Riet-Correa, F., Medeiros, R. M. T., Barbosa-Filho, J. M., & Mingatto, F. E. (2012). Mechanism for the uncoupling of oxidative phosphorylation by juliprosopine on rat brain mitochondria. *Toxicon*, *60*(8), 1355–1362.

Mair, G. R., Braks, J. A. M., Garver, L. S., Dimopoulos, G., Hall, N., Wiegant, J. C. A. G., Dirks, R. W., Khan, S. M., Janse, C. J., & Waters, A. P. (2006). Translational repression is essential for *Plasmodium* sexual development and mediated by a DDX6-type RNA helicase. *Science*, *313*(5787), 667–669.

Mair, G. R., Lasonder, E., Garver, L. S., Franke-Fayard, B. M. D., Carret, C. K., Wiegant, J. C. A. G., Dirks, R. W., Dimopoulos, G., Janse, C. J., & Waters, A. P. (2010). Universal features of post-transcriptional gene regulation are critical for *Plasmodium* zygote development. *PLOS Pathogens*, *6*(2), e1000767.

Malhotra, S., & Misra, K. (1983). New flavanones from *Prosopis juliflora* roots. *Planta Medica*, *47*(01), 46–48.

Malik, H. J., Raza, A., Amin, I., Scheffler, J. A., Scheffler, B. E., Brown, J. K., & Mansoor, S. (2016). RNAi-mediated mortality of the whitefly through transgenic expression of double-stranded RNA homologous to acetylcholinesterase and ecdysone receptor in tobacco plants. *Scientific Reports*, *6*, 38469.

Mambu, L., Martin, M.-T., Razafimahefa, D., Ramanitrahasimbola, D., Rasoanaivo, P., & Frappier, F. (2000). Spectral characterisation and antiplasmodial activity of bisbenzylisoquinolines from *Isolona ghesquiereina*. *Planta Med*, *66*(06), 537–540.

Mantel, P.-Y., Hoang, A. N., Goldowitz, I., Potashnikova, D., Hamza, B., Vorobjev, I., Ghiran, I., Toner, M., Irimia, D., Ivanov, A. R., Barteneva, N., & Marti, M. (2013). Malaria-infected erythrocyte-derived microvesicles mediate cellular communication within the parasite population and with the host immune system. *Cell Host &*

Microbe, *13*(5), 521–534.

Marcsisin, S. R., Sousa, J. C., Reichard, G. A., Caridha, D., Zeng, Q., Roncal, N., McNulty, R., Careagabarja, J., Sciotti, R. J., & Bennett, J. W. (2014). Tafenoquine and NPC-1161B require CYP 2D metabolism for anti-malarial activity: implications for the 8-aminoquinoline class of anti-malarial compounds. *Malaria Journal*, *13*(1), 2.

Mariath, I. R., Falcão, H. de S., Barbosa-Filho, J. M., Sousa, L. C. F. de, Tomaz, A. C. de A., Batista, L. M., Diniz, M. de F. F. M., Athayde-Filho, P. F., Tavares, J. F., Silva, M. S., & Cunha, E. V. L. da. (2009). Plants of the American continent with antimalarial activity. *Revista Brasileira de Farmacognosia*, *19*, 158–191.

Marques, S. R., Ramakrishnan, C., Carzaniga, R., Blagborough, A. M., Delves, M. J., Talman, A. M., & Sinden, R. E. (2015). An essential role of the basal body protein SAS-6 in *Plasmodium* male gamete development and malaria transmission. *Cellular Microbiology*, *17*(2), 191–206.

Marra, F., Salzman, J. R., & Ensom, M. H. H. (2003). Atovaquone–proguanil for prophylaxis and treatment of malaria. *Annals of Pharmacotherapy*, *37*(9), 1266–1275.

Marshall, S. J., Russell, P. F., Wright, C. W., Anderson, M. M., Phillipson, J. D., Kirby, G. C., Warhurst, D. C., & Schiff, P. L. (1994). *In vitro* antiplasmodial, antiamoebic, and cytotoxic activities of a series of bisbenzylisoquinoline alkaloids. *Antimicrobial Agents and Chemotherapy*, *38*(1), 96–103.

Martin, S. K., Jett, M., & Schneider, I. (1994). Correlation of phosphoinositide hydrolysis with exflagellation in the malaria microgametocyte. *The Journal of Parasitology*, 371–378.

Matasyoh, J. C., Talontsi, F. M., Ngoumfo, R. M., & Chepkorir, R. (2011). Larvicidal activity of acridone alkaloids from *Zanthoxylum leprieurii* against the malaria vector *Anopheles gambiae*. *The Natural Products Journal*, *1*(2), 121–124.

Mathias, D. K., Pastrana-mena, R., Ranucci, E., Tao, D., Ferruti, P., Ortega, C., Staples, G. O., Zaia, J., Takashima, E., Tsuboi, T., Borg, N. A., Verotta, L., & Dinglasan, R.

R. (2013). A small molecule glycosaminoglycan mimetic blocks *Plasmodium* invasion of the mosquito midgut. *PLoS Pathogens*, *9*(11), e1003757.

Matu, E. N., & van Staden, J. (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *Journal of Ethnopharmacology*, *87*(1), 35–41.

Maude, R. J., Woodrow, C. J., & White, L. J. (2010). Artemisinin antimalarials: Preserving the "Magic Bullet." *Drug Development Research*, *71*(1), 12–19.

Mbengue, A., Bhattacharjee, S., Pandharkar, T., Liu, H., Estiu, G., Stahelin, R. V, Rizk, S. S., Njimoh, D. L., Ryan, Y., & Chotivanich, K. (2015). A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. *Nature*, *520*(7549), 683–687.

McCarthy, J. S., Rückle, T., Djeriou, E., Cantalloube, C., Ter-Minassian, D., Baker, M., O'Rourke, P., Griffin, P., Marquart, L., & van Huijsduijnen, R. H. (2016). A phase II pilot trial to evaluate safety and efficacy of ferroquine against early *Plasmodium falciparum* in an induced blood-stage malaria infection study. *Malaria Journal*, *15*(1), 1–9.

McNamara, C. W., Lee, M. C. S., Lim, C. S., Lim, S. H., Roland, J., Nagle, A., Simon, O., Yeung, B. K. S., Chatterjee, A. K., McCormack, S. L., Manary, M. J., Zeeman, A.-M., Dechering, K. J., Kumar, T. R. S., Henrich, P. P., Gagaring, K., Ibanez, M., Kato, N., Kuhen, K. L., … Winzeler, E. A. (2013). Targeting *Plasmodium* PI(4)K to eliminate malaria. *Nature*, *504*(7479), 248–253.

McRobert, L., Taylor, C. J., Deng, W., Fivelman, Q. L., Cummings, R. M., Polley, S. D., Billker, O., & Baker, D. A. (2008). Gametogenesis in malaria parasites is mediated by the cGMP-dependent protein kinase. *PLOS Biology*, *6*(6), e139.

Meibalan, E., & Marti, M. (2017). Biology of malaria transmission. *Cold Spring Harbor Perspectives in Medicine*, *7*(3), a025452.

Meissner, P. E., Mandi, G., Coulibaly, B., Witte, S., Tapsoba, T., Mansmann, U., Rengelshausen, J., Schiek, W., Jahn, A., & Walter-Sack, I. (2006). Methylene blue for malaria in Africa: results from a dose-finding study in combination with

chloroquine. *Malaria Journal*, *5*(1), 1–5.

Meister, S., Agianian, B., Turlure, F., Relogio, A., Morlais, I., & Kafatos, F. C. (2009). *Anopheles gambiae* PGRPLC-mediated defense against bacteria modulates infections with malaria parasites. *PLoS Pathog*, *5*(8), e1000542.

Mekonnen, S. K., Aseffa, A., Berhe, N., Teklehaymanot, T., Clouse, R. M., Gebru, T., Medhin, G., & Velavan, T. P. (2014). Return of chloroquine-sensitive *Plasmodium falciparum* parasites and emergence of chloroquine-resistant *Plasmodium vivax* in Ethiopia. *Malaria Journal*, *13*(1), 244.

Mendes, A. M., Schlegelmilch, T., Cohuet, A., Awono-Ambene, P., De Iorio, M., Fontenille, D., Morlais, I., Christophides, G. K., Kafatos, F. C., & Vlachou, D. (2008). Conserved mosquito/parasite interactions affect development of *Plasmodium falciparum* in Africa. *PLoS Pathog*, *4*(5), e1000069.

Menon, V., Kapulu, M. C., Taylor, I., Jewell, K., Li, Y., Hill, F., Long, C. A., Miura, K., & Biswas, S. (2018). Assessment of antibodies induced by multivalent transmission-blocking malaria vaccines. *Frontiers in Immunology*, *8*, 1998.

Merschjohann, K., Sporer, F., Steverding, D., & Wink, M. (2001). *In vitro* effect of alkaloids on bloodstream forms of *Trypanosoma brucei* and *T. congolense*. *Planta Medica*, *67*(7), 623–627.

Meshnick, S. R. (2002). Artemisinin: mechanisms of action, resistance and toxicity. *International Journal for Parasitology*, *32*(13), 1655–1660.

Meshnick, S. R., & Dobson, M. J. (2001). The history of antimalarial drugs. In *Antimalarial chemotherapy* pp 15–26. Springer

Meyers, J. I., Pathikonda, S., Popkin-Hall, Z. R., Medeiros, M. C., Fuseini, G., Matias, A., Garcia, G., Overgaard, H. J., Kulkarni, V., & Reddy, V. P. (2016). Increasing outdoor host-seeking in *Anopheles gambiae* over 6 years of vector control on Bioko Island. *Malaria Journal*, *15*(1), 1–13.

Miao, J., Chen, Z., Wang, Z., Shrestha, S., Li, X., Li, R., & Cui, L. (2017). Sex-Specific biology of the human malaria parasite revealed from the proteomes of mature male and female gametocytes. *Molecular Cellular Proteomics*, *16*(4), 537 – 551.

Miao, J., Fan, Q., Cui, L., Li, J., Li, J., & Cui, L. (2006). The malaria parasite *Plasmodium falciparum* histones: Organization, expression, and acetylation. *Gene*, *369*, 53–65.

Miao, J., Fan, Q., Parker, D., Li, X., Li, J., & Cui, L. (2013). Puf mediates translation repression of transmission-blocking vaccine candidates in malaria parasites. *PLOS Pathogens*, *9*(4), e1003268.

Miao, J., Li, J., Fan, Q., Li, X., Li, X., & Cui, L. (2010). The Puf-family RNA-binding protein PfPuf2 regulates sexual development and sex differentiation in the malaria parasite *Plasmodium falciparum*. *Journal of Cell Science*, *123*(7), 1039–1049.

Michel, K., Suwanchaichinda, C., Morlais, I., Lambrechts, L., Cohuet, A., Awono-Ambene, P. H., Simard, F., Fontenille, D., Kanost, M. R., & Kafatos, F. C. (2006). Increased melanizing activity in *Anopheles gambiae* does not affect development of *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences*, *103*(45), 16858–16863.

Miguel-Blanco, C., Molina, I., Bardera, A. I., Díaz, B., de las Heras, L., Lozano, S., González, C., Rodrigues, J., Delves, M. J., Ruecker, A., Colmenarejo, G., Viera, S., Martínez-Martínez, M. S., Fernández, E., Baum, J., Sinden, R. E., & Herreros, E. (2017). Hundreds of dual-stage antimalarial molecules discovered by a functional gametocyte screen. *Nature Communications*, *8*(1), 15160.

Miotto, O., Almagro-Garcia, J., Manske, M., MacInnis, B., Campino, S., Rockett, K. A., Amaratunga, C., Lim, P., Suon, S., & Sreng, S. (2013). Multiple populations of artemisinin-resistant *Plasmodium falciparum* in Cambodia. *Nature Genetics*, *45*(6), 648–655.

Mishra, S., Nussenzweig, R. S., & Nussenzweig, V. (2012). Antibodies to *Plasmodium* circumsporozoite protein (CSP) inhibit sporozoite's cell traversal activity. *Journal of Immunological Methods*, *377*(1–2), 47–52.

Mithöfer, A., & Boland, W. (2012). Plant defense against herbivores: Chemical aspects. *Annual Review of Plant Biology*, *63*(1), 431–450.

Modrzynska, K., Pfander, C., Chappell, L., Yu, L., Suarez, C., Dundas, K., Gomes, A.

R., Goulding, D., Rayner, J. C., Choudhary, J., & Billker, O. (2017). A knockout screen of ApiAP2 genes reveals networks of interacting transcriptional regulators controlling the *Plasmodium* life cycle. *Cell Host & Microbe*, *21*(1), 11–22.

Mohamad, K., Hirasawa, Y., Litaudon, M., Awang, K., Hadi, A. H. A., Takeya, K., Ekasari, W., Widyawaruyanti, A., Zaini, N. C., & Morita, H. (2009). Ceramicines B–D, new antiplasmodial limonoids from *Chisocheton ceramicus*. *Bioorganic & Medicinal Chemistry*, *17*(2), 727–730.

Molina-Cruz, A., Garver, L. S., Alabaster, A., Bangiolo, L., Haile, A., Winikor, J., Ortega, C., van Schaijk, B. C. L., Sauerwein, R. W., Taylor-Salmon, E., & Barillas-Mury, C. (2013). The human malaria parasite Pfs47 gene mediates evasion of the mosquito immune system. *Science*, *340*(6135), 984 – 987.

Moon, H.-I. (2007). Antiplasmodial activity of ineupatorolides A from *Carpesium rosulatum*. *Parasitology Research*, *100*(5), 1147–1149.

Moon, H.-I., Jung, J.-C., & Lee, J. (2007). Antiplasmodial activity of triterpenoid isolated from whole plants of *Viola* genus from South Korea. *Parasitology Research*, *100*(3), 641–644.

Moon, R. W., Taylor, C. J., Bex, C., Schepers, R., Goulding, D., Janse, C. J., Waters, A. P., Baker, D. A., & Billker, O. (2009). A cyclic GMP signalling module that regulates gliding motility in a malaria parasite. *PLOS Pathogens*, *5*(9), e1000599.

Morais, S. K. R. de, Silva, S. G., Portela, C. N., Nunomura, S. M., Quignard, E. L. J., & Pohlit, A. M. (2007). Bioactive dihydroxyfuranonaphthoquinones from the bark of *Tabebuia incana* A.H. Gentry (Bignoniaceae) and HPLC analysis of commercial pau d'arco and certified *T. incana* bark infusions. *Acta Amazonica*, *37*, 99–102.

Moreira, L. A., & Jacobs-Lorena, M. (2003). Transgenic mosquitoes for malaria control: progresses and challenges. *Neotropical Entomology 32*, 531–536.

Moreno-García, M., Recio-Tótoro, B., Claudio-Piedras, F., & Lanz-Mendoza, H. (2014). Injury and immune response: applying the danger theory to mosquitoes. *Frontiers in Plant Science*, *5*, 451.

Moretti, C., Sauvain, M., Lavaud, C., Massiot, G., Bravo, J.-A., & Muñoz, V. (1998). A

novel antiprotozoal aminosteroid from *Saracha punctata*. *Journal of Natural Products*, *61*(11), 1390–1393.

Morita, H., Oshimi, S., Hirasawa, Y., Koyama, K., Honda, T., Ekasari, W., Indrayanto, G., & Zaini, N. C. (2007). Cassiarins A and B, novel antiplasmodial alkaloids from *Cassia siamea*. *Organic Letters*, *9*(18), 3691–3693.

Moshi, M. J., Kagashe, G. A. B., & Mbwambo, Z. H. (2005). Plants used to treat epilepsy by Tanzanian traditional healers. *Journal of Ethnopharmacology*, *97*(2), 327–336.

Moussavi, N., Malterud, K. E., Mikolo, B., Dawes, D., Chandre, F., Corbel, V., Massamba, D., Overgaard, H. J., & Wangensteen, H. (2015). Identification of chemical constituents of *Zanthoxylum heitzii* stem bark and their insecticidal activity against the malaria mosquito *Anopheles gambiae*. *Parasites & Vectors*, *8*, 503.

Moyo, P., Botha, M. E., Nondaba, S., Niemand, J., Maharaj, V. J., Eloff, J. N., Louw, A. I., & Birkholtz, L. (2016). *In vitro* inhibition of *Plasmodium falciparum* early and late stage gametocyte viability by extracts from eight traditionally used South African plant species. *Journal of Ethnopharmacology*, *185*, 235–242.

Msellemu, D., Namango, H. I., Mwakalinga, V. M., Ntamatungiro, A. J., Mlacha, Y., Mtema, Z. J., Kiware, S., Lobo, N. F., Majambere, S., & Dongus, S. (2016). The epidemiology of residual *Plasmodium falciparum* malaria transmission and infection burden in an African city with high coverage of multiple vector control measures. *Malaria Journal*, *15*(1), 288.

Muema, J. M., Bargul, J. L., Njeru, S. N., Onyango, J. O., & Imbahale, S. S. (2017). Prospects for malaria control through manipulation of mosquito larval habitats and olfactory-mediated behavioural responses using plant-derived compounds. *Parasites & Vectors*, *10*(1), 184.

Muema, J. M., Bargul, J. L., Nyanjom, S. G., Mutunga, J. M., & Njeru, S. N. (2016). Potential of *Camellia sinensis* proanthocyanidins-rich fraction for controlling malaria mosquito populations through disruption of larval development. *Parasites*

& *Vectors*, *9*(1), 512.

Muema, J. M., Nyanjom, S. G., Mutunga, J. M., Njeru, S. N., & Bargul, J. L. (2017). Green tea proanthocyanidins cause impairment of hormone-regulated larval development and reproductive fitness via repression of juvenile hormone acid methyltransferase, insulin-like peptide and cytochrome P450 genes in *Anopheles gambiae* sensu stricto. *PLOS ONE*, *12*(3), e0173564.

Muganga, R., Angenot, L., Tits, M., & Frederich, M. (2010). Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria. *Journal of Ethnopharmacology*, *128*(1), 52–57.

Muhammad, I., Bedir, E., Khan, S. I., Tekwani, B. L., Khan, I. A., Takamatsu, S., Pelletier, J., & Walker, L. A. (2004). A new antimalarial quassinoid from *Simaba orinocensis*. *Journal of Natural Products*, *67*(5), 772–777.

Muhia, D. K., Swales, C. A., Deng, W., Kelly, J. M., & Baker, D. A. (2001). The gametocyte-activating factor xanthurenic acid stimulates an increase in membrane-associated guanylyl cyclase activity in the human malaria parasite *Plasmodium falciparum*. *Molecular Microbiology*, *42*(2), 553–560.

Muller, G. C., Junnila, A., Traore, M. M., Traore, S. F., Doumbia, S., Sissoko, F., Dembele, S. M., Schlein, Y., Arheart, K. L., Revay, E. E., Kravchenko, V. D., Witt, A., & Beier, J. C. (2017). The invasive shrub *Prosopis juliflora* enhances the malaria parasite transmission capacity of *Anopheles* mosquitoes: a habitat manipulation experiment. *Malaria Journal*, *16*(1), 237.

Müller, K., Matuschewski, K., & Silvie, O. (2011). The Puf-family RNA-binding protein Puf2 controls sporozoite conversion to liver stages in the malaria parasite. *PloS One*, *6*(5), e19860.

Murakami, C., Fukamiya, N., Tamura, S., Okano, M., Bastow, K. F., Tokuda, H., Mukainaka, T., Nishino, H., & Lee, K.-H. (2004). Multidrug-resistant cancer cell susceptibility to cytotoxic quassinoids, and cancer chemopreventive effects of quassinoids and canthin alkaloids. *Bioorganic & Medicinal Chemistry*, *12*(18), 4963–4968.

Mutembei, J., Kareru, P., Njonge, F., Peter, G., Kutima, H., Karanja, J., & Kimani, D. (2015). *In-vivo* anthelmintic evaluation of a processed herbal drug from *Entada leptostachya* (Harms) and *Prosopis juliflora* (Sw.)(DC) against gastrointestinal nematodes in sheep. *IOSR Journal of Polymer and Textile Engineering*, *2*(1), 6–10.

Muthana, K. D., & Arora, G. D. (1983). *Prosopis juliflora (Swartz) DC, a fast growing tree to bloom the desert*. Central Arid Zone Research Institute; Jodhpur.

Mutunga, J. M., Ma, M., Chen, Q.-H., Hartsel, J. A., Wong, D. M., Ding, S., Totrov, M., Carlier, P. R., & Bloomquist, J. R. (2019). Mosquito acetylcholinesterase as a target for novel phenyl-substituted carbamates. *International Journal of Environmental Research and Public Health*, *16*(9), 1500.

Mwangi, E., & Swallow, B. (2005). Invasion of *Prosopis juliflora* and local livelihoods: Case study from the lake Baringo area of Kenya. *Nairobi, Kenya: World Agroforestry Centre*.

Mwanza, S., Joshi, S., Nambozi, M., Chileshe, J., Malunga, P., Kabuya, J.-B. B., Hachizovu, S., Manyando, C., Mulenga, M., & Laufer, M. (2016). The return of chloroquine-susceptible *Plasmodium falciparum* malaria in Zambia. *Malaria Journal*, *15*(1), 584.

Mwesigwa, J., Achan, J., Di Tanna, G. L., Affara, M., Jawara, M., Worwui, A., Hamid-Adiamoh, M., Kanuteh, F., Ceesay, S., Bousema, T., Drakeley, C., Grietens, K. P., Lindsay, S. W., Van geertruyden, J.-P., & D'Alessandro, U. (2017). Residual malaria transmission dynamics varies across The Gambia despite high coverage of control interventions. *PLOS ONE*, *12*(11), e0187059.

Na-Bangchang, K., Muhamad, P., Ruaengweerayut, R., Chaijaroenkul, W., & Karbwang, J. (2013). Identification of resistance of *Plasmodium falciparum* to artesunate-mefloquine combination in an area along the Thai-Myanmar border: integration of clinico-parasitological response, systemic drug exposure, and in vitro parasite sensitivity. *Malaria Journal*, *12*(1), 263.

Naissant, B., Dupuy, F., Duffier, Y., Lorthiois, A., Duez, J., Scholz, J., Buffet, P., Merckx, A., Bachmann, A., & Lavazec, C. (2016). *Plasmodium falciparum*

STEVOR phosphorylation regulates host erythrocyte deformability enabling malaria parasite transmission. *Blood, 127*(24), e42 LP-e53.

Namiki, T., Niwa, R., Sakudoh, T., Shirai, K., Takeuchi, H., & Kataoka, H. (2005). Cytochrome P450 CYP307A1/Spook: A regulator for ecdysone synthesis in insects. *Biochemical and Biophysical Research Communications*, *337*(1), 367–374.

Naoshima-Ishibashi, Y., Iwagami, M., Kawazu, S., Looareesuwan, S., & Kano, S. (2007). Analyses of cytochrome b mutations in *Plasmodium falciparum* isolates in Thai-Myanmar border. *Travel Medicine and Infectious Disease*, *5*(2), 132–134.

Neveu, G., & Lavazec, C. (2019). Erythrocyte membrane makeover by *Plasmodium falciparum* gametocytes. *Frontiers in Microbiology*, *10*, 2652.

Nguta, J. M., Mbaria, J. M., Gakuya, D. W., Gathumbi, P. K., & Kiama, S. G. (2010). Traditional antimalarial phytotherapy remedies used by the South Coast community, Kenya. *Journal of Ethnopharmacology*, *131*(2), 256–267.

Ngwa, Che J, Kiesow, M. J., Papst, O., Orchard, L. M., Filarsky, M., Rosinski, A. N., Voss, T. S., Llinás, M., & Pradel, G. (2017). Transcriptional profiling defines histone acetylation as a regulator of gene expression during human-to-mosquito transmission of the malaria parasite *Plasmodium falciparum*. *Frontiers in Cellular and Infection Microbiology*, *7*, 320.

Ngwa, Che Julius, Scheuermayer, M., Mair, G. R., Kern, S., Brügl, T., Wirth, C. C., Aminake, M. N., Wiesner, J., Fischer, R., Vilcinskas, A., & Pradel, G. (2013). Changes in the transcriptome of the malaria parasite *Plasmodium falciparum* during the initial phase of transmission from the human to the mosquito. *BMC Genomics*, *14*(1), 256.

Nibret, E., Ashour, M. L., Rubanza, C. D., & Wink, M. (2010). Screening of some Tanzanian medicinal plants for their trypanocidal and cytotoxic activities. *Phytotherapy Research*, *24*(6), 945–947.

Nilsson, S. K., Childs, L. M., Buckee, C., & Marti, M. (2015). Targeting human transmission biology for malaria elimination. *PLOS Pathogens*, *11*(6), e1004871.

Niu, G., Franc A, C., Zhang, G., Roobsoong, W., Nguitragool, W., Wang, X.,

Prachumsri, J., Butler, N. S., & Li, J. (2017). The fibrinogen-like domain of FREP1 protein is a broad-spectrum malaria transmission-blocking vaccine antigen. *The Journal of Biological Chemistry*, *292*(28), 11960–11969.

Niu, G., Wang, B., Zhang, G., King, J. B., Cichewicz, R. H., & Li, J. (2015). Targeting mosquito FREP1 with a fungal metabolite blocks malaria transmission. *Scientific Reports*, *5*(1), 14694.

Niwa, Y. S., & Niwa, R. (2014). Neural control of steroid hormone biosynthesis during development in the fruit fly *Drosophila melanogaster*. *Genes & Genetic Systems*, *89*(1), 27–34.

Niwa, Y. S., & Niwa, R. (2016). Transcriptional regulation of insect steroid hormone biosynthesis and its role in controlling timing of molting and metamorphosis. *Development, Growth & Differentiation*, *58*(1), 94–105.

Njeru, S. N., Obonyo, M. A., Nyambati, S. O., & Ngari, S. M. (2015). Bioactivity of *Cissampelos pareira* medicinal plant against *Mycobacterium tuberculosis*. *J Pharmacog & Phytochem*, *3*, 167–173.

Nkumama, I. N., O'Meara, W. P., & Osier, F. H. A. (2016). Changes in malaria epidemiology in Africa and new challenges for elimination. *Trends in Parasitology*, *33*(2), 128–140.

Noor, A. M., Kinyoki, D. K., Mundia, C. W., Kabaria, C. W., Mutua, J. W., Alegana, V. A., Fall, I. S., & Snow, R. W. (2014). The changing risk of *Plasmodium falciparum* malaria infection in Africa: 2000-2013: a spatial and temporal analysis of transmission intensity. *The Lancet*, *383*(9930), 1739–1747.

Novicki, A., & Weeks, J. C. (1993). Organization of the larval pre-ecdysis motor pattern in the tobacco hornworm, Manduca sexta. *Journal of Comparative Physiology A*, *173*(2), 151–162.

Nsango, S. E., Pompon, J., Xie, T., Rademacher, A., Fraiture, M., Thoma, M., Awono-Ambene, P. H., Moyou, R. S., Morlais, I., & Levashina, E. A. (2013). AP-1/Fos-TGase2 axis mediates wounding-induced *Plasmodium falciparum* killing in *Anopheles gambiae*. *Journal of Biological Chemistry*, *288*(22), 16145–16154.

Ntie-Kang, F., Onguéné, P. A., Lifongo, L. L., Ndom, J. C., Sippl, W., & Mbaze, L. M. (2014). The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. *Malaria Journal*, *13*(1), 81.

Nyamoita, M. G. (2013). Toxicity of individual and blends of pure phytoecdysteroids isolated from *Vitex schiliebenii* and *Vitex payos* against *Anopheles gambiae* ss larvae. *World Journal of Organic Chemistry*, *1*(1), 1–5.

Nyasembe, V. O., Cheseto, X., Kaplan, F., Foster, W. a., Teal, P. E. a., Tumlinson, J. H., Borgemeister, C., & Torto, B. (2015). The invasive American weed *Parthenium hysterophorus* can negatively impact malaria control in Africa. *Plos One*, *10*(9), e0137836.

O'Neill, P. M., Barton, V. E., Ward, S. A., & Chadwick, J. (2012). 4-Aminoquinolines: Chloroquine, Amodiaquine and next-generation analogues BT - treatment and prevention of malaria: Antimalarial drug chemistry, action and use (H. M. Staines & S. Krishna (Eds.); pp. 19–44. Springer Basel.

Olivieri, A., Camarda, G., Bertuccini, L., Van De Vegte-Bolmer, M., Luty, A. J. F., Sauerwein, R., & Alano, P. (2009). The *Plasmodium falciparum* protein Pfg27 is dispensable for gametocyte and gamete production, but contributes to cell integrity during gametocytogenesis. *Molecular Microbiology*, *73*(2), 180–193.

Olliaro, P., & Wells, T. N. C. (2009). The global portfolio of new antimalarial medicines under development. *Clinical Pharmacology & Therapeutics*, *85*(6), 584–595.

Omole, R. A. (2011). Anti-malarial activity and phytochemical studies of *Cissampelos mucronata* and *Stephania abyssinica*. *Unpublished Master Thesis, Kenyatta University*.

Onambele, L. A., Riepl, H., Fischer, R., Pradel, G., Prokop, A., & Aminake, M. N. (2015). Synthesis and evaluation of the antiplasmodial activity of tryptanthrin derivatives. *International Journal for Parasitology. Drugs and Drug Resistance*, *5*(2), 48–57.

Onguéné, A., Onguéné, P. A., Ntie-kang, F., Lifongo, L. L., Ndom, J. C., & Sippl, W.

(2013). The potential of anti-malarial compounds derived from African medicinal plants . Part I : A pharmacological evaluation of alkaloids and terpenoids The potential of anti-malarial compounds derived from African medicinal plants . Part I : A pharmacological. *Malaria Journal*, *12*, 449.

Onyeibor, O., Croft, S. L., Dodson, H. I., Feiz-Haddad, M., Kendrick, H., Millington, N. J., Parapini, S., Phillips, R. M., Seville, S., & Shnyder, S. D. (2005). Synthesis of some cryptolepine analogues, assessment of their antimalarial and cytotoxic activities, and consideration of their antimalarial mode of action. *Journal of Medicinal Chemistry*, *48*(7), 2701–2709.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Simons, A. (2009). Agroforestree Database: a tree reference and selection guide. Version 4. *Agroforestree Database: A Tree Reference and Selection Guide. Version 4.*

Oshimi, S., Takasaki, A., Hirasawa, Y., Hosoya, T., Awang, K., Hadi, A. H. A., Ekasari, W., Widyawaruyanti, A., & Morita, H. (2009). Delaumonones A and B, new antiplasmodial quassinoids from *Laumoniera bruceadelpha*. *Chemical and Pharmaceutical Bulletin*, *57*(8), 867–869.

Osta, M. A., Christophides, G. K., & Kafatos, F. C. (2004). Effects of mosquito genes on *Plasmodium* development. *Science*, *303*(5666), 2030–2032.

Ou, Q., Zeng, J., Yamanaka, N., Brakken-Thal, C., O'Connor, M. B., & King-Jones, K. (2016). The insect prothoracic gland as a model for steroid hormone biosynthesis and regulation. *Cell Reports*, *16*(1), 247–262.

Ouédraogo, A. L., Bousema, T., de Vlas, S. J., Cuzin-Ouattara, N., Verhave, J.-P., Drakeley, C., Luty, A. J. F., & Sauerwein, R. (2010). The plasticity of *Plasmodium falciparum* gametocytaemia in relation to age in Burkina Faso. *Malaria Journal*, *9*(1), 281.

Overgaard, H. J., Sirisopa, P., Mikolo, B., Malterud, K. E., Wangensteen, H., Zou, Y.-F., Paulsen, B. S., Massamba, D., Duchon, S., & Corbel, V. (2014). Insecticidal activities of bark, leaf and seed extracts of *Zanthoxylum heitzii* against the African malaria vector *Anopheles gambiae*. *Molecules*, *19*(12), 21276–21290.

Oxborough, R. M. (2016). Trends in US President's Malaria Initiative-funded indoor residual spray coverage and insecticide choice in sub-Saharan Africa (2008–2015): urgent need for affordable, long-lasting insecticides. *Malaria Journal*, *15*(1), 146.

Pace, T., Olivieri, A., Sanchez, M., Albanesi, V., Picci, L., Kiamos, I. S., Janse, C. J., Waters, A. P., Pizzi, E., & Ponzi, M. (2006). Set regulation in asexual and sexual *Plasmodium* parasites reveals a novel mechanism of stage-specific expression. *Molecular Microbiology*, *60*(4), 870–882.

Packard, R. M. (2014). The origins of antimalarial-drug resistance. *New England Journal of Medicine*, *371*(5), 397–399.

Padonou, G. G., Gbedjissi, G., Yadouleton, A., Azondekon, R., Razack, O., Oussou, O., Gnanguenon, V., Rock, A., Sezonlin, M., & Akogbeto, M. (2012). Decreased proportions of indoor feeding and endophily in *Anopheles gambiae* sl populations following the indoor residual spraying and insecticide-treated net interventions in Benin (West Africa). *Parasites & Vectors*, *5*(1), 262.

Pakpour, N., Akman-Anderson, L., Vodovotz, Y., & Luckhart, S. (2013). The effects of ingested mammalian blood factors on vector arthropod immunity and physiology. *Microbes and Infection*, *15*(3), 243–254.

Palacharla, R. C., Uthukam, V., Manoharan, A., Ponnamaneni, R. K., Padala, N. P., Boggavarapu, R. K., Bhyrapuneni, G., Ajjala, D. R., & Nirogi, R. (2017). Inhibition of cytochrome P450 enzymes by saturated and unsaturated fatty acids in human liver microsomes, characterization of enzyme kinetics in the presence of bovine serum albumin (0.1 and 1.0% w/v) and *in vitro–in vivo* extrapolation of hepatic clearanc. *European Journal of Pharmaceutical Sciences*, *101*, 80–89.

Pang, Y.-P., Brimijoin, S., Ragsdale, D. W., Zhu, K. Y., & Suranyi, R. (2012). Novel and viable acetylcholinesterase target site for developing effective and environmentally safe insecticides. *Current Drug Targets*, *13*(4), 471–482.

Pankotai, T., Popescu, C., Martin, D., Grau, B., Zsindely, N., Bodai, L., Tora, L., Ferrus, A., & Boros, I. (2010). Genes of the ecdysone biosynthesis pathway are regulated by the dATAC histone acetyltransferase complex in *Drosophila*. *Molecular and*

Cellular Biology, *30*(17), 4254–4266.

Papers, J. B. C., Doi, M., Li, F., Templeton, T. J., Popov, V., Comer, J. E., Tsuboi, T., Torii, M., & Vinetz, J. M. (2004). *Plasmodium* ookinete-secreted proteins secreted through a common micronemal pathway are targets of blocking malaria transmission. *Journal of Biological Chemistry*, *279*(25), 26635–26644.

Paquet, T., Manach, C. Le, Cabrera, D. G., Younis, Y., Henrich, P. P., Abraham, T. S., Lee, M. C. S., Basak, R., Ghidelli-disse, S., Lafuente-monasterio, M. J., Bantscheff, M., Ruecker, A., Blagborough, A. M., Zakutansky, S. E., Zeeman, A., White, K. L., Shackleford, D. M., Mannila, J., Morizzi, J., … Chibale, K. (2017). Antimalarial efficacy of MMV390048, an inhibitor of *Plasmodium* phosphatidylinositol 4-kinase. *Science Translational Medicine*, *9*(387), eaad9735.

Pasiecznik, N. (1999). *Prosopis-pest or providence, weed or wonder tree?*

Pastrana-mena, R., Mathias, D. K., Delves, M., Rajaram, K., King, J. G., Yee, R., Trucchi, B., Verotta, L., & Dinglasan, R. R. (2016). A malaria transmission-blocking (+)-Usnic acid derivative prevents *Plasmodium* zygote-to-ookinete maturation in the mosquito midgut. *ACS Chem Biol*, *11*(12), 3461–3472.

Patzewitz, E.-M., Guttery, D. S., Poulin, B., Ramakrishnan, C., Ferguson, D. J., Wall, R. J., Brady, D., Holder, A. A., Szöőr, B., & Tewari, R. (2013). An ancient protein phosphatase, SHLP1, is critical to microneme development in *Plasmodium* ookinetes and parasite transmission. *Cell Reports*, *3*(3), 622–629.

Pavela, R., & Govindarajan, M. (2017). The essential oil from *Zanthoxylum monophyllum* a potential mosquito larvicide with low toxicity to the non-target fish *Gambusia affinis*. *Journal of Pest Science*, *90*(1), 369–378.

Peatey, C. L., Skinner-adams, T. S., Dixon, M. W. A., Mccarthy, J. S., Gardiner, D. L., & Trenholme, K. R. (2017). Effect of antimalarial drugs on *Plasmodium falciparum gametocytes*. *Journal of Infectious Diseases*, *200*, 6–9.

Perumalsamy, H., Jang, M. J., Kim, J.-R., Kadarkarai, M., & Ahn, Y.-J. (2015). Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species. *Parasites*

& Vectors, *8*(1), 237.

Peters, W., Robinson, B. L., & Milhous, W. K. (1993). The chemotherapy of rodent malaria. LI. Studies on a new 8-aminoquinoline, WR 238,605. *Annals of Tropical Medicine & Parasitology*, *87*(6), 547–552.

Peterson, T. M. L., Gow, A. J., & Luckhart, S. (2007). Nitric oxide metabolites induced in *Anopheles stephensi* control malaria parasite infection. *Free Radical Biology & Medicine*, *42*(1), 132–142.

Phillips, M. A., Lotharius, J., Marsh, K., White, J., Dayan, A., White, K. L., Njoroge, J. W., El Mazouni, F., Lao, Y., & Kokkonda, S. (2015). A long-duration dihydroorotate dehydrogenase inhibitor (DSM265) for prevention and treatment of malaria. *Science Translational Medicine*, *7*(296), 296ra111-296ra111.

Phrutivorapongkul, A., Ichino, C., Ruangrungsi, N., Ishiyama, A., Sekiguchi, H., Namatame, M., Kiyohara, H., Otoguro, K., Yamada, H., & Omura, S. (2006). Anti-Plasmodial activity of bisbenzylisoquinoline alkaloids from *Michelia figo* leaves. *Journal of Health Research*, *20*(2), 121–128.

Pietri, J. E., Pakpour, N., Napoli, E., Song, G., Pietri, E., Potts, R., Cheung, K. W., Walker, G., Riehle, M. A., Starcevich, H., Giulivi, C., Lewis, E. E., & Luckhart, S. (2016). Two insulin-like peptides differentially regulate malaria parasite infection in the mosquito through effects on intermediary metabolism. *Biochemical Journal*, *473*(20), 3487–3503.

Pillay, P., Maharaj, V. J., & Smith, P. J. (2008). Investigating South African plants as a source of new antimalarial drugs. *Journal of Ethnopharmacology*, *119*(3), 438–454.

Pinilla, Y. T., C P Lopes, S., S Sampaio, V., Andrade, F. S., Melo, G. C., Orfanó, A. S., Secundino, N. F. C., Guerra, M. G. V. B., Lacerda, M. V. G., Kobylinski, K. C., Escobedo-Vargas, K. S., López-Sifuentes, V. M., Stoops, C. A., Baldeviano, G. C., Tarning, J., Vasquez, G. M., Pimenta, P. F. P., & Monteiro, W. M. (2018). Promising approach to reducing Malaria transmission by ivermectin: Sporontocidal effect against *Plasmodium vivax* in the South American vectors *Anopheles aquasalis* and *Anopheles darlingi*. *PLoS Neglected Tropical Diseases*, *12*(2),

e0006221

Plouffe, D. M., Wree, M., Du, A. Y., Meister, S., Li, F., Patra, K., Lubar, A., Okitsu, S. L., Flannery, E. L., Kato, N., Tanaseichuk, O., Comer, E., Zhou, B., Kuhen, K., Zhou, Y., Leroy, D., Schreiber, S. L., Scherer, C. A., Vinetz, J., & Winzeler, E. A. (2016). High-throughput assay and discovery of small molecules that interrupt malaria transmission. *Cell Host & Microbe*, *19*(1), 114–126.

Pohlit, A. M., Lima, R. B., Frausin, G., Silva, L. F., Lopes, S. C., Moraes, C. B., Cravo, P., Lacerda, M. V, Siqueira, A. M., Freitas-Junior, L. H., & Costa, F. T. (2013). Amazonian plant natural products: Perspectives for discovery of new antimalarial drug leads. *Molecules 18*, 8.

Pooda, H. S., Rayaisse, J.-B., Hien, D. F. de S., Lefèvre, T., Yerbanga, S. R., Bengaly, Z., Dabiré, R. K., Belem, A. M. G., Sidibé, I., Solano, P., & Mouline, K. (2015). Administration of ivermectin to peridomestic cattle: a promising approach to target the residual transmission of human malaria. *Malaria Journal*, *13 Suppl 1*, 496.

Pooda, S. H., Mouline, K., De Meeûs, T., Bengaly, Z., & Solano, P. (2013). Decrease in survival and fecundity of *Glossina palpalis* gambiensis vanderplank 1949 (Diptera: Glossinidae) fed on cattle treated with single doses of ivermectin. *Parasites & Vectors*, *6*(1), 165.

Prabha, D. S., Dahms, H.-U., & Malliga, P. (2014). Pharmacological potentials of phenolic compounds from *Prosopis* spp.-a. *Journal of Coastal Life Medicine*, *2*(11), 918–924.

Pradel, G. (2007). Proteins of the malaria parasite sexual stages: expression, function and potential for transmission blocking strategies. *Parasitology*, *134*(14), 1911.

Project, M. *P. falciparum* C. (2016). Genomic epidemiology of artemisinin resistant malaria. *ELife*, *5*, e08714.

Rahman, A. A., Samoylenko, V., Jacob, M. R., Sahu, R., Jain, S. K., Khan, S. I., Tekwani, B. L., & Muhammad, I. (2011). Antiparasitic and antimicrobial indolizidines from the leaves of *Prosopis glandulosa* var. glandulosa. *Planta Med*, *77*(14), 1639–1643.

Rahman, M. M., Islam, M. A., Khondkar, P., & Gray, A. I. (2005). Alkaloids and lignans from *Zanthoxylum budrunga* (Rutaceae). *Biochemical Systematics and Ecology*, *1*(33), 91–96.

Raine, J. D., Ecker, A., Mendoza, J., Tewari, R., Stanway, R. R., & Sinden, R. E. (2007). Female inheritance of malarial lap genes is essential for mosquito transmission. *PLoS Pathogens*, *3*(3), e30.

Ramanitrahasimbola, D., Rasoanaivo, P., Ratsimamanga-Urverg, S., Federici, E., Palazzino, G., Galeffi, C., & Nicoletti, M. (2001). Biological activities of the plant-derived bisindole voacamine with reference to malaria. *Phytotherapy Research*, *15*(1), 30–33.

Ramazani, A., Zakeri, S., Sardari, S., Khodakarim, N., & Djadidt, N. D. (2010). *In vitro* and *in vivo* anti-malarial activity of *Boerhavia elegans* and *Solanum surattense*. *Malaria Journal*, *9*(1), 124.

Ramdani, G., Naissant, B., Thompson, E., Breil, F., Lorthiois, A., Dupuy, F., Cummings, R., Duffier, Y., Corbett, Y., Mercereau-Puijalon, O., Vernick, K., Taramelli, D., Baker, D. A., Langsley, G., & Lavazec, C. (2015). cAMP-signalling regulates gametocyte-infected erythrocyte deformability required for malaria parasite transmission. *PLOS Pathogens*, *11*(5), e1004815.

Ramírez, I., Carabot, A., Meléndez, P., Carmona, J., Jimenez, M., Patel, A. V, Crabb, T. A., Blunden, G., Cary, P. D., & Croft, S. L. (2003). Cissampeloflavone, a chalcone-flavone dimer from *Cissampelos pareira*. *Phytochemistry*, *64*(2), 645–647.

Ramphul, U. N., Garver, L. S., Molina-Cruz, A., Canepa, G. E., & Barillas-Mury, C. (2015). *Plasmodium falciparum* evades mosquito immunity by disrupting JNK-mediated apoptosis of invaded midgut cells. *Proceedings of the National Academy of Sciences*, *112*(5), 1273 – 1280.

Ranson, H., & Lissenden, N. (2016). Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. *Trends in Parasitology*, *32*(3), 187–196.

Rao, P. N., Santos, J. M., Pain, A., Templeton, T. J., & Mair, G. R. (2016). Translational

repression of the cpw-wpc gene family in the malaria parasite *Plasmodium*. *Parasitology International*, *65*(5, Part A), 463–471.

Reddy, M. R., Overgaard, H. J., Abaga, S., Reddy, V. P., Caccone, A., Kiszewski, A. E., & Slotman, M. A. (2011). Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malar J*, *10*(1), 184.

Reininger, L., Garcia, M., Tomlins, A., Müller, S., & Doerig, C. (2012). The *Plasmodium falciparum*, Nima-related kinase Pfnek-4: a marker for asexual parasites committed to sexual differentiation. *Malaria Journal*, *11*, 250.

Reininger, L., Tewari, R., Fennell, C., Holland, Z., Goldring, D., Ranford-cartwright, L., Billker, O., & Doerig, C. (2009). An essential role for the *Plasmodium* Nek-2 Nima-related protein kinase in the sexual development of malaria parasites. *Journal of Biological Chemistry*, *284* (31), 20858-20868.

Riehle, M. M., Xu, J., Lazzaro, B. P., Rottschaefer, S. M., Coulibaly, B., Sacko, M., Niare, O., Morlais, I., Traore, S. F., & Vernick, K. D. (2008). *Anopheles gambiae* APL1 is a family of variable LRR proteins required for Rel1-mediated protection from the malaria parasite, *Plasmodium berghei*. *PLOS ONE*, *3*(11), e3672.

Riveron, J. M., Watsenga, F., Irving, H., Irish, S. R., & Wondji, C. S. (2018). High *Plasmodium* infection rate and reduced bed net efficacy in multiple insecticide-resistant malaria vectors in Kinshasa, Democratic Republic of Congo. *The Journal of Infectious Diseases*, *217*(2), 320–328.

Robinson, A., Busula, A. O., Voets, M. A., Beshir, K. B., Caulfield, J. C., Powers, S. J., Verhulst, N. O., Winskill, P., Muwanguzi, J., Birkett, M. A., Smallegange, R. C., Masiga, D. K., Mukabana, W. R., Sauerwein, R. W., Sutherland, C. J., Bousema, T., Pickett, J. A., Takken, W., Logan, J. G., & de Boer, J. G. (2018). *Plasmodium*-associated changes in human odor attract mosquitoes. *Proceedings of the National Academy of Sciences*, *115*(18), E4209-E4218.

Rodgers, F. H., Gendrin, M., Wyer, C. A. S., & Christophides, G. K. (2017). Microbiota-induced peritrophic matrix regulates midgut homeostasis and prevents systemic

infection of malaria vector mosquitoes. *PLoS Pathogens*, *13*(5), e1006391.

Rodrigues, J., Oliveira, G. A., Kotsyfakis, M., Dixit, R., Molina-Cruz, A., Jochim, R., & Barillas-Mury, C. (2012). An epithelial serine protease, AgESP, is required for Plasmodium invasion in the mosquito *Anopheles gambiae*. *PLOS ONE*, *7*(4), e35210.

Rodríguez, M. del C., Martínez-Barnetche, J., Alvarado-Delgado, A., Batista, C., Argotte-Ramos, R. S., Hernández-Martínez, S., González Cerón, L., Torres, J. A., Margos, G., & Rodríguez, M. H. (2007). The surface protein Pvs25 of *Plasmodium vivax* ookinetes interacts with calreticulin on the midgut apical surface of the malaria vector *Anopheles albimanus*. *Molecular and Biochemical Parasitology*, *153*(2), 167–177.

Romoli, O., & Gendrin, M. (2018). The tripartite interactions between the mosquito, its microbiota and *Plasmodium*. *Parasites & Vectors*, *11*(1), 200.

Rono, M. K., Nyonda, M. A., Simam, J. J., Ngoi, J. M., Mok, S., Kortok, M. M., Abdullah, A. S., Elfaki, M. M., Waitumbi, J. N., El-Hassan, I. M., Marsh, K., Bozdech, Z., & Mackinnon, M. J. (2018). Adaptation of *Plasmodium falciparum* to its transmission environment. *Nature Ecology & Evolution*, *2*(2), 377–387.

Ross, A., Killeen, G., & Smith, T. (2006). Relationships between host infectivity to mosquitoes and asexual parasite density in *Plasmodium falciparum*. *The American Journal of Tropical Medicine and Hygiene*, *75*(2_suppl), 32–37.

Ross, R. (1897). On some peculiar pigmented cells found in two mosquitos fed on malarial blood. *British Medical Journal*, *2*(1929), 1786.

Rottmann, M., McNamara, C., Yeung, B. K. S., Lee, M. C. S., Zou, B., Russell, B., Seitz, P., Plouffe, D. M., Dharia, N. V, Tan, J., Cohen, S. B., Spencer, K. R., González-Páez, G. E., Lakshminarayana, S. B., Goh, A., Suwanarusk, R., Jegla, T., Schmitt, E. K., Beck, H.-P., … Diagana, T. T. (2010). Spiroindolones, a potent compound class for the treatment of malaria. *Science*, *329*(5996), 1175–1180.

Rts, S. (2014). Clinical Trials Partnership. Efficacy and safety of the RTS, S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized,

controlled trial in children and young infants at 11 African sites. *PLoS Med*, *11*(7), e1001685.

RTS, S.C.T.P. (2012). A Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Infants. *New England Journal of Medicine*, *367*(24), 2284-2295.

RTS, S. C. T. P. (2015). Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *The Lancet*, *386*(9988), 31–45.

Ruangweerayut, R., Looareesuwan, S., Hutchinson, D., Chauemung, A., Banmairuroi, V., & Na-Bangchang, K. (2008). Assessment of the pharmacokinetics and dynamics of two combination regimens of fosmidomycin-clindamycin in patients with acute uncomplicated *falciparum* malaria. *Malaria Journal*, *7*, 225.

Rukunga, G. M., Gathirwa, J. W., Omar, S. A., Muregi, F. W., Muthaura, C. N., Kirira, P. G., Mungai, G. M., & Kofi-Tsekpo, W. M. (2009). Anti-plasmodial activity of the extracts of some Kenyan medicinal plants. *Journal of Ethnopharmacology*, *121*(2), 282–285.

Rukunga, G. M., Muregi, F. W., Tolo, F. M., Omar, S. A., Mwitari, P., Muthaura, C. N., Omlin, F., Lwande, W., Hassanali, A., Githure, J., Iraqi, F. W., Mungai, G. M., Kraus, W., & Kofi-Tsekpo, W. M. (2007). The antiplasmodial activity of spermine alkaloids isolated from *Albizia gummifera*. *Fitoterapia*, *78*(7), 455–459.

Russell, T. L., Beebe, N. W., Bugoro, H., Apairamo, A., Chow, W. K., Cooper, R. D., Collins, F. H., Lobo, N. F., & Burkot, T. R. (2016). Frequent blood feeding enables insecticide-treated nets to reduce transmission by mosquitoes that bite predominately outdoors. *Malaria Journal*, *15*(1), 156.

Russell, T. L., Beebe, N. W., Cooper, R. D., Lobo, N. F., & Burkot, T. R. (2013). Successful malaria elimination strategies require interventions that target changing vector behaviours. *Malaria Journal*, *12*(1), 1–5.

Russell, T. L., Govella, N. J., Azizi, S., Drakeley, C. J., Kachur, S. P., & Killeen, G. F. (2011). Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania.

Malaria Journal, *10*(1), 80.

Sacconnay, L., Angleviel, M., Randazzo, G. M., Marçal Ferreira Queiroz, M., Ferreira Queiroz, E., Wolfender, J.-L., Carrupt, P.-A., & Nurisso, A. (2014). Computational studies on sirtuins from *Trypanosoma cruzi*: Structures, conformations and interactions with phytochemicals. *PLOS Neglected Tropical Diseases*, *8*(2), e2689.

Saini, R. P., Raman, V., Dhandapani, G., Malhotra, E. V., Sreevathsa, R., Kumar, P. A., Sharma, T. R., & Pattanayak, D. (2018). Silencing of HaAce1 gene by host-delivered artificial microRNA disrupts growth and development of *Helicoverpa armigera*. *PLOS ONE*, *13*(3), e0194150.

Sala, K. A., Nishiura, H., Upton, L. M., Zakutansky, S. E., Delves, M. J., Iyori, M., Mizutani, M., Sinden, R. E., Yoshida, S., & Blagborough, A. M. (2015). The *Plasmodium berghei* sexual stage antigen PSOP12 induces anti-malarial transmission blocking immunity both *in vivo* and *in vitro*. *Vaccine*, *33*(3), 437–445.

Samoylenko, V., Ashfaq, M. K., Jacob, M. R., Tekwani, B. L., Khan, S. I., Manly, S. P., Joshi, V. C., Walker, L. A., & Muhammad, I. (2009). Indolizidine, antiinfective and antiparasitic compounds from *Prosopis glandulosa* var. glandulosa. *Journal of Natural Products*, *72*(1), 92–98.

Sampaio, V. de S., Rivas, G. B. da S., Kobylinski, K., Pinilla, Y. T., Pimenta, P. F. P., Lima, J. B. P., Bruno, R. V., Lacerda, M. V. G., & Monteiro, W. M. (2017). What does not kill it makes it weaker: effects of sub-lethal concentrations of ivermectin on the locomotor activity of *Anopheles aquasalis*. *Parasites & Vectors*, *10*(1), 623.

Sanders, N. G., Sullivan, D. J., Mlambo, G., Dimopoulos, G., & Tripathi, A. K. (2014). Gametocytocidal screen identifies novel chemical classes with *Plasmodium falciparum* transmission blocking activity. *PLoS ONE*, *9*(8), e105817.

Sandeu, M. M., Abate, L., Tchioffo, M. T., Bayibéki, A. N., Awono-Ambéné, P. H., Nsango, S. E., Chesnais, C. B., Dinglasan, R. R., de Meeûs, T., & Morlais, I. (2016). Impact of exposure to mosquito transmission-blocking antibodies on *Plasmodium falciparum* population genetic structure. *Infection, Genetics and Evolution*, *45*, 138–144.

Santos, J. M., Egarter, S., Zuzarte-Luis, V., Kumar, H., Moreau, C. A., Kehrer, J., Pinto, A., da Costa, M., Franke-Fayard, B., & Janse, C. J. (2017). Malaria parasite LIMP protein regulates sporozoite gliding motility and infectivity in mosquito and mammalian hosts. *Elife*, *6*, e24109.

Santos, J. M., Kehrer, J., Franke-Fayard, B., Frischknecht, F., Janse, C. J., & Mair, G. R. (2015). The *Plasmodium* palmitoyl-S-acyl-transferase DHHC2 is essential for ookinete morphogenesis and malaria transmission. *Scientific Reports*, *5*, 16034.

Saraiva, R. G., Huitt-Roehl, C. R., Tripathi, A., Cheng, Y.-Q., Bosch, J., Townsend, C. A., & Dimopoulos, G. (2018). *Chromobacterium* spp. mediate their anti-*Plasmodium* activity through secretion of the histone deacetylase inhibitor romidepsin. *Scientific Reports*, *8*(1), 6176.

Sasaki, H., Sekiguchi, H., Sugiyama, M., & Ikadai, H. (2017). *Plasmodium berghei* Cap93, a novel oocyst capsule-associated protein, plays a role in sporozoite development. *Parasites & Vectors*, *10*(1), 399.

Sathiya, M., & Muthuchelian, K. (2010). Evaluation of antioxidant and antitumor potentials of *Prosopis juliflora* DC. leaves *in vitro*. *Pharmacology Online*, *2,* 328–343.

Saunders, D. L., Vanachayangkul, P., & Lon, C. (2014). Dihydroartemisinin–piperaquine failure in Cambodia. *New England Journal of Medicine*, *371*(5), 484–485.

Saxena, S., Pant, N., Jain, D. C., & Bhakuni, R. S. (2003). Antimalarial agents from plant sources. *Current Science*, *85*.

Schlitzer, M. (2007). Malaria chemotherapeutics part I: History of antimalarial drug development, currently used therapeutics, and drugs in clinical development. *ChemMedChem: Chemistry Enabling Drug Discovery*, *2*(7), 944–986.

Schneider, M. P., Liu, B., Glock, P., Suttie, A., Mchugh, E., Andrew, D., Batinovic, S., Williamson, N., Hanssen, E., Mcmillan, P., Hliscs, M., Tilley, L., & Dixon, M. W. A. (2017). Disrupting assembly of the inner membrane complex blocks *Plasmodium falciparum* sexual stage development. *PLoS Pathogens*, 13(10),

e1006659.

Schneider, P., Bousema, J. T., Gouagna, L. C., Otieno, S., Van De Vegte-Bolmer, M., Omar, S. A., & Sauerwein, R. W. (2007). Submicroscopic *Plasmodium falciparum* gametocyte densities frequently result in mosquito infection. *The American Journal of Tropical Medicine and Hygiene*, *76*(3), 470–474.

Schneider, P., Rund, S. S. C., Smith, N. L., Prior, K. F., O'Donnell, A. J., & Reece, S. E. (2018). Adaptive periodicity in the infectivity of malaria gametocytes to mosquitoes. *Proceedings. Biological Sciences*, *285*(1888), 20181876.

Schubiger, M., Wade, A. A., Carney, G. E., Truman, J. W., & Bender, M. (1998). *Drosophila* EcR-B ecdysone receptor isoforms are required for larval molting and for neuron remodeling during metamorphosis. *Development*, *125*(11), 2053–2062.

Schwikkard, S., & van Heerden, F. R. (2002). Antimalarial activity of plant metabolites. *Natural Product Reports*, *19*(6), 675–692.

Sebastian, S., Brochet, M., Collins, M. O., Schwach, F., Jones, M. L., Goulding, D., Rayner, J. C., Choudhary, J. S., & Billker, O. (2012). A *Plasmodium* calcium-dependent protein kinase controls zygote development and transmission by translationally activating repressed mRNAs. *Cell Host & Microbe*, *12*(1–10), 9–19.

Semwal, D. K., Semwal, R. B., Vermaak, I., & Viljoen, A. (2014). From arrow poison to herbal medicine–the ethnobotanical, phytochemical and pharmacological significance of *Cissampelos* (Menispermaceae). *Journal of Ethnopharmacology*, *155*(2), 1011–1028.

Senn, M., Gunzenhauser, S., Brun, R., & Séquin, U. (2007). Antiprotozoal polyacetylenes from the Tanzanian medicinal plant *Cussonia zimmermannii*. *Journal of Natural Products*, *70*(10), 1565–1569.

Senthilkumar, N., Varma, P., & Gurusubramanian, G. (2009). Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, *Anopheles stephensi* (Liston). *Parasitology Research*, *104*(2), 237–244.

Severo, M. S., & Levashina, E. A. (2014). Mosquito defenses against *Plasmodium* parasites. *Current Opinion in Insect Science*, *3*, 30–36.

Sharma, S. K., Parasuraman, P., Kumar, G., Surolia, N., & Surolia, A. (2007). Green tea catechins potentiate triclosan binding to enoyl-ACP reductase from *Plasmodium falciparum* (PfENR). *Journal of Medicinal Chemistry*, *50*(4), 765–775.

Shaw, P. J., Chaotheing, S., Kaewprommal, P., Piriyapongsa, J., Wongsombat, C., Suwannakitti, N., Koonyosying, P., Uthaipibull, C., Yuthavong, Y., & Kamchonwongpaisan, S. (2015). *Plasmodium* parasites mount an arrest response to dihydroartemisinin, as revealed by whole transcriptome shotgun sequencing (RNA-seq) and microarray study. *BMC Genomics*, *16*(1), 830.

Sheen, W.-S., Tsai, I.-L., Teng, C.-M., & Chen, I.-S. (1994). Nor-neolignan and phenyl propanoid from *Zanthoxylum ailanthoides*. *Phytochemistry*, *36*(1), 213–215.

Sheen, W.-S., Tsai, I.-L., Teng, C.-M., Ko, F.-N., & Chen, I.-S. (1996). Indolopyridoquinazoline alkaloids with antiplatelet aggregation activity from *Zanthoxylum integrifoliolum*. *Planta Medica*, *62*(02), 175–176.

Shiao, S.-H., Whitten, M. M. A., Zachary, D., Hoffmann, J. A., & Levashina, E. A. (2006). Fz2 and Cdc42 mediate melanization and actin polymerization but are dispensable for *Plasmodium* killing in the mosquito midgut. *PLOS Pathogens*, *2*(12), e133.

Shimada-Niwa, Y., & Niwa, R. (2014). Serotonergic neurons respond to nutrients and regulate the timing of steroid hormone biosynthesis in Drosophila. *Nature Communications*, *5*, 5778.

Shimizu, S., Osada, Y., Kanazawa, T., Tanaka, Y., & Arai, M. (2010). Suppressive effect of azithromycin on *Plasmodium berghei* mosquito stage development and apicoplast replication. *Malaria Journal*, *9*, 73.

Shrestha, S., Li, X., Ning, G., Miao, J., & Cui, L. (2016). The RNA-binding protein Puf1 functions in the maintenance of gametocytes in *Plasmodium falciparum*. *Journal of Cell Science*, *129*(16), 3144–3152.

Siden-Kiamos, I., Ganter, M., Kunze, A., Hliscs, M., Steinbüchel, M., Mendoza, J., Sinden, R. E., Louis, C., & Matuschewski, K. (2011). Stage-specific depletion of myosin A supports an essential role in motility of malarial ookinetes. *Cellular*

Microbiology, *13*(12), 1996–2006.

Silva, V. D. A., Pitanga, B. P. S., Nascimento, R. P., Souza, C. S., Coelho, P. L. C., Menezes-Filho, N., Silva, A. M. M., Costa, M. de F. D., El-Bachá, R. S., Velozo, E. S., & Costa, S. L. (2013). Juliprosopine and juliprosine from *Prosopis juliflora* leaves induce mitochondrial damage and cytoplasmic vacuolation on cocultured glial cells and neurons. *Chemical Research in Toxicology*, *26*(12), 1810–1820.

Silvestrini, F., Bozdech, Z., Lanfrancotti, A., Giulio, E. Di, Bultrini, E., Picci, L., deRisi, J. L., Pizzi, E., & Alano, P. (2005). Genome-wide identification of genes upregulated at the onset of gametocytogenesis in *Plasmodium falciparum*. *Molecular and Biochemical Parasitology*, *143*(1), 100–110.

Silvestrini, F., Lasonder, E., Olivieri, A., Camarda, G., van Schaijk, B., Sanchez, M., Younis Younis, S., Sauerwein, R., & Alano, P. (2010). Protein export marks the early phase of gametocytogenesis of the human malaria parasite *Plasmodium falciparum*. *Molecular Cellular Proteomics*, *9*(7), 1437– 1448.

Simões, M. L., Mlambo, G., Tripathi, A., Dong, Y., & Dimopoulos, G. (2017). Immune regulation of *Plasmodium* is *Anopheles* species specific and infection intensity dependent. *MBio*, *8*(5), e01631-17.

Simon, N., Lasonder, E., Scheuermayer, M., Kuehn, A., Tews, S., Fischer, R., Zipfel, P. F., Skerka, C., & Pradel, G. (2013). Malaria parasites co-opt human factor H to prevent complement-mediated lysis in the mosquito midgut. *Cell Host & Microbe*, *13*(1), 29–41.

Sinden, R. (2010). A biologist's perspective on malaria vaccine development. *Human Vaccines*, *6*(1), 3–11.

Sinden, R E, & Smalley, M. E. (1979). Gametocytogenesis of *Plasmodium falciparum* in vitro: the cell-cycle. *Parasitology*, *79*(2), 277–296.

Sinden, Robert E. (2017). Developing transmission-blocking strategies for malaria control. *PLoS Pathogens*, *13*(7), e1006336.

Sinden, Robert E, Carter, R., Drakeley, C., & Leroy, D. (2012). The biology of sexual development of *Plasmodium* : the design and implementation of transmission-

blocking strategies. *Malaria Journal*, *11*, 70.

Singh, S., & Verma, S. K. (2012). Study of the distribution profile of piperidine alkaloids in various parts of *Prosopis juliflora* by the application of Direct Analysis in Real Time Mass Spectrometry (DART-MS). *Natural Products and Bioprospecting*, *2*(5), 206–209.

Sinha, A., Hughes, K. R., Modrzynska, K. K., Otto, T. D., Pfander, C., Dickens, N. J., Religa, A. a, Bushell, E., Graham, A. L., Cameron, R., Kafsack, B. F. C., Williams, A. E., Llinás, M., Berriman, M., Billker, O., & Waters, A. P. (2014). A cascade of DNA-binding proteins for sexual commitment and development in *Plasmodium*. *Nature*, *507*(7491), 253–257.

Sirignano, C., Snene, A., Rigano, D., Tapanelli, S., Formisano, C., Luciano, P., El Mokni, R., Hammami, S., Tenoh, A. R., Habluetzel, A., & Taglialatela-Scafati, O. (2017). Angeloylated germacranolides from *Daucus virgatus* and their *Plasmodium* transmission blocking activity. *Journal of Natural Products*, *80*(10), 2787–2794.

Sirmah, P., Dumarcay, S., Masson, E., & Gerardin, P. (2009). Unusual amount of (−)-mesquitol from the heartwood of *Prosopis juliflora*. *Natural Product Research*, *23*(2), 183–189.

Sissoko, F., Junnila, A., Traore, M. M., Traore, S. F., Doumbia, S., Dembele, S. M., Schlein, Y., Traore, A. S., Gergely, P., Xue, R.-D., Arheart, K. L., Revay, E. E., Kravchenko, V. D., Beier, J. C., & Müller, G. C. (2019). Frequent sugar feeding behavior by *Aedes aegypti* in Bamako, Mali makes them ideal candidates for control with attractive toxic sugar baits (ATSB). *PLOS ONE*, *14*(6), e0214170.

Siwo, G. H., Smith, R. S., Tan, A., Button-Simons, K. A., Checkley, L. A., & Ferdig, M. T. (2015). An integrative analysis of small molecule transcriptional responses in the human malaria parasite *Plasmodium falciparum*. *BMC Genomics*, *16*(1), 1–17.

Sleebs, B. E., Lopaticki, S., Marapana, D. S., Neill, M. T. O., Rajasekaran, P., Whitehead, L. W., Lowes, K. N., Barfod, L., Hviid, L., Gazdik, M., Gu, S., Shaw, P. J., Hodder, A. N., Smith, B. J., Cowman, A. F., & Boddey, J. A. (2014). Inhibition of plasmepsin V activity demonstrates its essential role in protein export ,

PfEMP1 display, and survival of malaria parasites. *PLoS Biology*, *12*(7), e1001897.

Smit, M. R., Ochomo, E. O., Aljayyoussi, G., Kwambai, T. K., Abong'o, B. O., Chen, T., Bousema, T., Slater, H. C., Waterhouse, D., Bayoh, N. M., Gimnig, J. E., Samuels, A. M., Desai, M. R., Phillips-Howard, P. A., Kariuki, S. K., Wang, D., Ward, S. A., & ter Kuile, F. O. (2018). Safety and mosquitocidal efficacy of high-dose ivermectin when co-administered with dihydroartemisinin-piperaquine in Kenyan adults with uncomplicated malaria (IVERMAL): a randomised, double-blind, placebo-controlled trial. *The Lancet Infectious Diseases*, *18*(6), 615–626.

Smith, D. L., Battle, K. E., Hay, S. I., Barker, C. M., Scott, T. W., & McKenzie, F. E. (2012). Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. *PLOS Pathogens*, *8*(4), e1002588.

Smith, R. C., Vega-Rodríguez, J., & Jacobs-Lorena, M. (2014). The *Plasmodium* bottleneck: malaria parasite losses in the mosquito vector. *Memórias Do Instituto Oswaldo Cruz*, *109*(5), 644–661.

Song, X., Wang, M., Dong, L., Zhu, H., & Wang, J. (2018). PGRP-LD mediates *A. stephensi* vector competency by regulating homeostasis of microbiota-induced peritrophic matrix synthesis. *PLoS Pathogens*, 14(2), e1006899.

Sonoiki, E., Ng, C. L., Lee, M. C. S., Guo, D., Zhang, Y.-K., Zhou, Y., Alley, M. R. K., Ahyong, V., Sanz, L. M., Lafuente-Monasterio, M. J., Dong, C., Schupp, P. G., Gut, J., Legac, J., Cooper, R. A., Gamo, F.-J., DeRisi, J., Freund, Y. R., Fidock, D. A., & Rosenthal, P. J. (2017). A potent antimalarial benzoxaborole targets a *Plasmodium falciparum* cleavage and polyadenylation specificity factor homologue. *Nature Communications*, *8*(1), 14574.

Sood, R., Raut, R., Tyagi, P., Pareek, P. K., Barman, T. K., Singhal, S., Shirumalla, R. K., Kanoje, V., Subbarayan, R., & Rajerethinam, R. (2015). *Cissampelos pareira* Linn: Natural source of potent antiviral activity against all four dengue virus serotypes. *PLoS Neglected Tropical Diseases*, *9*(12), e0004255.

Soreq, H., & Seidman, S. (2001). Acetylcholinesterase — new roles for an old actor. *Nature Reviews Neuroscience*, *2*, 294.

Spillman, N. J., Allen, R. J. W., Mcnamara, C. W., Yeung, B. K. S., Winzeler, E. A., Diagana, T. T., & Kirk, K. (2013). Na+ regulation in the malaria parasite *Plasmodium falciparum* involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. *Cell Host and Microbe*, *13*(2), 227–237.

Spindler-Barth, M., Schmidt, H., Drews, U., & Spindler, K.-D. (1988). Increase in activity of acetylcholinesterase by 20-OH-ecdysone in a *Chironomus tentans* cell line. *Roux's Archives of Developmental Biology*, *197*(6), 366–369.

Sreenivasamurthy, S. K., Dey, G., Ramu, M., Kumar, M., Gupta, M. K., Mohanty, A. K., Harsha, H. C., Sharma, P., Kumar, N., Pandey, A., Kumar, A., & Prasad, T. S. K. (2013). A compendium of molecules involved in vector-pathogen interactions pertaining to malaria. *Malaria Journal*, *12*(1), 1–7.

Srinivasan, P., Fujioka, H., & Jacobs-Lorena, M. (2008). PbCap380, a novel oocyst capsule protein, is essential for malaria parasite survival in the mosquito. *Cellular Microbiology*, *10*(6), 1304–1312.

Srivastava, A., Philip, N., Hughes, K. R., Georgiou, K., MacRae, J. I., Barrett, M. P., Creek, D. J., McConville, M. J., & Waters, A. P. (2016). Stage-specific changes in *Plasmodium* metabolism required for differentiation and adaptation to different host and vector environments. *PLOS Pathogens*, *12*(12), e1006094.

Stanczyk, N. M., Mescher, M. C., & De Moraes, C. M. (2017). Effects of malaria infection on mosquito olfaction and behavior: extrapolating data to the field. *Current Opinion in Insect Science*, *20*, 7–12.

Steinbuechel, M., & Matuschewski, K. (2009). Role for the *Plasmodium* sporozoite-specific transmembrane protein S6 in parasite motility and efficient malaria transmission. *Cellular Microbiology*, *11*(2), 279–288.

Stone, C. M., Witt, A. B. R., Walsh, G. C., Foster, W. A., & Murphy, S. T. (2018). Would the control of invasive alien plants reduce malaria transmission? A review. *Parasites & Vectors*, *11*(1), 76.

Stork, G., Niu, D., Fujimoto, A., Koft, E. R., Balkovec, J. M., Tata, J. R., & Dake, G. R. (2001). The first stereoselective total synthesis of quinine. *Journal of the American*

Chemical Society, *123*(14), 3239–3242.

Straschil, U., Talman, A. M., Ferguson, D. J. P., Bunting, K. A., Xu, Z., Bailes, E., Sinden, R. E., Holder, A. A., Smith, E. F., & Coates, J. C. (2010). The Armadillo repeat protein PF16 is essential for flagellar structure and function in *Plasmodium* male gametes. *PLoS One*, *5*(9), e12901.

Suárez-Cortés, P., Gambara, G., Favia, A., Palombi, F., Alano, P., & Filippini, A. (2017). Ned - 19 inhibition of parasite growth and multiplication suggests a role for NAADP mediated signalling in the asexual development of *Plasmodium falciparum*. *Malaria Journal*, *16*(1), 366.

Sugahara, R., Tanaka, S., & Shiotsuki, T. (2017). RNAi-mediated knockdown of SPOOK reduces ecdysteroid titers and causes precocious metamorphosis in the desert locust *Schistocerca gregaria*. *Developmental Biology*, *429*(1), 71–80.

Sun, W., Huang, X., Li, H., Tawa, G., Fisher, E., Tanaka, T. Q., Shinn, P., & Huang, W. (2017). Novel lead structures with both *Plasmodium falciparum* gametocytocidal and asexual blood stage activity identified from high throughput compound screening. *Malaria Journal*, 16, 147.

Sun, W., Tanaka, T. Q., Magle, C. T., Huang, W., Southall, N., Huang, R., Dehdashti, S. J., McKew, J. C., Williamson, K. C., & Zheng, W. (2014). Chemical signatures and new drug targets for gametocytocidal drug development. *Scientific Reports*, *4*, 3743.

Surachetpong, W., Pakpour, N., Cheung, K. W., & Luckhart, S. (2011). Reactive oxygen species-dependent cell signaling regulates the mosquito immune response to *Plasmodium falciparum*. *Antioxidants & Redox Signaling*, *14*(6), 943–955.

Sylla, M., Kobylinski, K. C., Gray, M., Chapman, P. L., Sarr, M. D., Rasgon, J. L., & Foy, B. D. (2010). Mass drug administration of ivermectin in south-eastern Senegal reduces the survivorship of wild-caught, blood fed malaria vectors. *Malaria Journal*, *9*(1), 365.

Tabuti, J. R. S., Kukunda, C. B., & Waako, P. J. (2010). Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related

ailments in Uganda. *Journal of Ethnopharmacology*, *127*(1), 130–136.

Takala-Harrison, S., Jacob, C. G., Arze, C., Cummings, M. P., Silva, J. C., Dondorp, A. M., Fukuda, M. M., Hien, T. T., Mayxay, M., & Noedl, H. (2015). Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. *Journal of Infectious Diseases*, *211*(5), 670–679.

Takken, W., & Knols, B. G. J. (1999). Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annual Review of Entomology*, *44*(1), 131–157.

Talaat, K. R., Ellis, R. D., Hurd, J., Hentrich, A., Gabriel, E., Hynes, N. A., Rausch, K. M., Zhu, D., Muratova, O., Herrera, R., Anderson, C., Jones, D., Aebig, J., Brockley, S., MacDonald, N. J., Wang, X., Fay, M. P., Healy, S. A., Durbin, A. P., … Duffy, P. E. (2016). Safety and immunogenicity of Pfs25-EPA/Alhydrogel®, a transmission blocking vaccine against *Plasmodium falciparum*: An open label study in malaria naïve adults. *PLOS ONE*, *11*(10), e0163144.

Talontsi, F. M., Matasyoh, J. C., Ngoumfo, R. M., & Chepkorir, R. (2011). Mosquito larvicidal activity of alkaloids from *Zanthoxylum lemairei* against the malaria vector *Anopheles gambiae*. *Pesticide Biochemistry and Physiology*, *99*(1), 82–85.

Talundzic, E., Chenet, S. M., Goldman, I. F., Patel, D. S., Nelson, J. A., Plucinski, M. M., Barnwell, J. W., & Udhayakumar, V. (2015). Genetic analysis and species specific amplification of the artemisinin resistance-associated kelch propeller domain in *P. falciparum* and *P. vivax*. *PloS One*, *10*(8), e0136099.

Tamburini, R., Albuquerque, E. X., Daly, J. W., & Kauffman, F. C. (1982). Inhibition of calcium-dependent ATPase from sarcoplasmic reticulum by a new class of indolizidine alkaloids, Pumiliotoxins A, B, and 251D. *Journal of Neurochemistry*, *37*(3), 775–780.

Tao, D., Ubaida-Mohien, C., Mathias, D. K., King, J. G., Pastrana-Mena, R., Tripathi, A., Goldowitz, I., Graham, D. R., Moss, E., Marti, M., & Dinglasan, R. R. (2014). Sex-partitioning of the *Plasmodium falciparum* stage V gametocyte proteome provides insight into *falciparum*-specific cell biology. *Molecular & Cellular Proteomics*, *13*(10), 2705–2724.

Tapanelli, S., Chianese, G., Lucantoni, L., Yerbanga, R. S., Habluetzel, A., & Taglialatela-Scafati, O. (2016). Transmission blocking effects of neem (*Azadirachta indica*) seed kernel limonoids on *Plasmodium berghei* early sporogonic development. *Fitoterapia*, *114*, 122–126.

Tarique, M., Ahmad, M., Ansari, A., & Tuteja, R. (2013). *Plasmodium falciparum* DOZI, an RNA helicase interacts with eIF4E. *Gene*, *522*(1), 46–59.

Taylor, D. K., Avery, T. D., Greatrex, B. W., Tiekink, E. R. T., Macreadie, I. G., Macreadie, P. I., Humphries, A. D., Kalkanidis, M., Fox, E. N., & Klonis, N. (2004). Novel endoperoxide antimalarials: synthesis, heme binding, and antimalarial activity. *Journal of Medicinal Chemistry*, *47*(7), 1833–1839.

Taylor, S. M., Parobek, C. M., DeConti, D. K., Kayentao, K., Coulibaly, S. O., Greenwood, B. M., Tagbor, H., Williams, J., Bojang, K., & Njie, F. (2015). Absence of putative artemisinin resistance mutations among *Plasmodium falciparum* in sub-Saharan Africa: a molecular epidemiologic study. *The Journal of Infectious Diseases*, *211*(5), 680–688.

Templeton, T. J., Keister, D. B., Muratova, O., Procter, J. L., & Kaslow, D. C. (1998). Adherence of erythrocytes during exflagellation of *Plasmodium falciparum* microgametes is dependent on erythrocyte surface sialic acid and glycophorins. *The Journal of Experimental Medicine*, *187*(10), 1599–1609.

Tenywa, F. C., Kambagha, A., Saddler, A., & Maia, M. F. (2017). The development of an ivermectin-based attractive toxic sugar bait (ATSB) to target *Anopheles arabiensis*. *Malaria Journal*, *16*(1), 338.

Tewari, R., Dorin, D., Moon, R., Doerig, C., & Billker, O. (2005). An atypical mitogen-activated protein kinase controls cytokinesis and flagellar motility during male gamete formation in a malaria parasite. *Molecular Microbiology*, *58*(5), 1253–1263.

Tewari, R., Straschil, U., Bateman, A., Böhme, U., Cherevach, I., Gong, P., Pain, A., & Billker, O. (2010). The systematic functional analysis of *Plasmodium* protein kinases identifies essential regulators of mosquito transmission. *Cell Host &*

Microbe, *8*(4), 377–387.

Thiem, D. A., Sneden, A. T., Khan, S. I., & Tekwani, B. L. (2005). Bisnortriterpenes from *Salacia madagascariensis*. *Journal of Natural Products*, *68*(2), 251–254.

Thompson, J., Fernandez-Reyes, D., Sharling, L., Moore, S. G., Eling, W. M., Kyes, S. A., Newbold, C. I., Kafatos, F. C., Janse, C. J., & Waters, A. P. (2007). *Plasmodium* cysteine repeat modular proteins 1–4: complex proteins with roles throughout the malaria parasite life cycle. *Cellular Microbiology*, *9*(6), 1466–1480.

Tibúrcio, M., Dixon, M. W. A., Looker, O., Younis, S. Y., Tilley, L., & Alano, P. (2015). Specific expression and export of the *Plasmodium falciparum* Gametocyte EXported Protein-5 marks the gametocyte ring stage. *Malaria Journal*, *14*, 334.

Tibúrcio, M., Sauerwein, R., Lavazec, C., & Alano, P. (2015). Erythrocyte remodeling by *Plasmodium falciparum* gametocytes in the human host interplay. *Trends in Parasitology*, *31*(6), 270–278.

Tiwary, M., Naik, S. N., Tewary, D. K., Mittal, P. K., & Yadav, S. (2007). Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. *Journal of Vector Borne Diseases*, *44*(3), 198.

Tomé, H. V., Pascini, T. V, Dângelo, R. A., Guedes, R. N., & Martins, G. F. (2014). Survival and swimming behavior of insecticide-exposed larvae and pupae of the yellow fever mosquito *Aedes aegypti*. *Parasites & Vectors*, *7*(1), 195.

Toutant, J.-P. (1989). Insect acetylcholinesterase: Catalytic properties, tissue distribution and molecular forms. *Progress in Neurobiology*, *32*(5), 423–446.

Tran, P. N., Brown, S. H. J., Mitchell, T. W., Matuschewski, K., McMillan, P. J., Kirk, K., Dixon, M. W. A., & Maier, A. G. (2014). A female gametocyte-specific ABC transporter plays a role in lipid metabolism in the malaria parasite. *Nature Communications*, *5*, 4773.

Tran, P. N., Brown, S. H. J., Rug, M., Ridgway, M. C., Mitchell, T. W., & Maier, A. G. (2016). Changes in lipid composition during sexual development of the malaria parasite *Plasmodium falciparum*. *Malaria Journal*, *15*, 73.

Trape, J.-F., & Rogier, C. (1996). Combating malaria morbidity and mortality by reducing transmission. *Parasitology Today*, *12*(6), 236–240.

Trapotsi, M.-A., Hosseini-Gerami, L., & Bender, A. (2022). Computational analyses of mechanism of action (MoA): data, methods and integration. *RSC Chemical Biology*, *3*, 170–200.

Trenholme, K., Marek, L., Duffy, S., Pradel, G., Fisher, G., Hansen, F. K., Skinner-adams, T. S., Butterworth, A., Ngwa, J., Moecking, J., Goodman, C. D., Mcfadden, G. I., Sumanadasa, S. D. M., Fairlie, D. P., Avery, V. M., Kurz, T., & Andrews, T. (2014). Lysine acetylation in sexual stage malaria parasites is a target for antimalarial small molecules. *Antimicrobial Agents and Chemotherapy*, *58*(7), 3666–3678.

Trudel, R. E., & Bomblies, A. (2011). Larvicidal effects of Chinaberry (*Melia azederach*) powder on *Anopheles arabiensis* in Ethiopia. *Parasites & Vectors*, *4*(1), 72.

Truman, J W, Talbot, W. S., Fahrbach, S. E., & Hogness, D. S. (1994). Ecdysone receptor expression in the CNS correlates with stage-specific responses to ecdysteroids during *Drosophila* and *Manduca* development. *Development*, *120*(1), 219 – 234.

Truman, James W. (1996). Steroid receptors and nervous system metamorphosis in insects. *Developmental Neuroscience*, *18*(1–2), 87–101.

Truman, James W, & Reiss, S. E. (1995). Neuromuscular metamorphosis in the moth *Manduca sexta*: hormonal regulation of synapses loss and remodeling. *Journal of Neuroscience*, *15*(7), 4815–4826.

Tshibangu, J. N., Wright, A. D., & König, G. M. (2003). HPLC isolation of the anti-plasmodially active bisbenzylisoquinone alkaloids present in roots of *Cissampelos mucronata*. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *14*(1), 13–22.

Tusting, L. S., Thwing, J., Sinclair, D., Fillinger, U., Gimnig, J., Bonner, K. E., Bottomley, C., & Lindsay, S. W. (2013). Mosquito larval source management for

controlling malaria. *The Cochrane Database of Systematic Reviews*, *8*, CD008923.

Uddin Ahmad, V., & Sultana, A. (1989). A terpenoid diketone from the leaves of *Prosopis juliflora*. *Phytochemistry*, *28*(1), 278–279.

Ukegbu, C. V., Giorgalli, M., Yassine, H., Ramirez, J. L., Taxiarchi, C., Barillas-Mury, C., Christophides, G. K., & Vlachou, D. (2017). *Plasmodium berghei* P47 is essential for ookinete protection from the *Anopheles gambiae* complement-like response. *Scientific Reports*, *7*(1), 6026.

Usanga, E. A., O'Brien, E., & Luzzato, L. (1986). Mitotic inhibitors arrest the growth of *Plasmodium falciparum*. *FEBS Letters*, *209*(1), 23–27.

Valecha, N., Adak, T., Bagga, A. K., Asthana, O. P., Srivastava, J. S., Joshi, H., & Sharma, V. P. (2001). Comparative antirelapse efficacy of CDRI compound 80/53 (Bulaquine) vs primaquine in double blind clinical trial. *Current Science*, 561–563.

van Biljon, R., van Wyk, R., Painter, H. J., Orchard, L., Reader, J., Niemand, J., Llinás, M., & Birkholtz, L.-M. (2019). Hierarchical transcriptional control regulates *Plasmodium falciparum* sexual differentiation. *BMC Genomics*, *20*(1), 920.

van der Watt, M. E., Reader, J., Churchyard, A., Nondaba, S. H., Lauterbach, S. B., Niemand, J., Abayomi, S., van Biljon, R. A., Connacher, J. I., van Wyk, R. D. J., Le Manach, C., Paquet, T., González Cabrera, D., Brunschwig, C., Theron, A., Lozano-Arias, S., Rodrigues, J. F. I., Herreros, E., Leroy, D., … Birkholtz, L.-M. (2018). Potent *Plasmodium falciparum* gametocytocidal compounds identified by exploring the kinase inhibitor chemical space for dual active antimalarials. *Journal of Antimicrobial Chemotherapy*, *73*(5), 1279–1290.

van Dijk, M. R., Janse, C. J., Thompson, J., Waters, A. P., Braks, J. A. M., Dodemont, H. J., Stunnenberg, H. G., van Gemert, G.-J., Sauerwein, R. W., & Eling, W. (2001). A central role for P48/45 in malaria parasite male gamete fertility. *Cell*, *104*(1), 153–164.

van Pelt-Koops, J. C., Pett, H. E., Graumans, W., van der Vegte-Bolmer, M., van Gemert, G. J., Rottmann, M., Yeung, B. K., Diagana, T. T., & Sauerwein, R. W. (2012). The spiroindolone drug candidate NITD609 potently inhibits

gametocytogenesis and blocks *Plasmodium falciparum* transmission to anopheles mosquito vector. *Antimicrob Agents Chemother*, *56*(7), 3544–3548.

van Schaijk, B.C.L., Kumar, T.R.S., Vos, M.W., Richman, A., van Gemert, G.-J., Li, T., Eappen, A.G., Williamson, K.C., Morahan, B.J., Fishbaugher, M., Kennedy M., Camargo, N., Khan, S.M., Janse, C.J., Sim, K.L., Hoffman, S.L., Kappe, S.H.I., Sauerwein, R.W., Fidock D.A., & Vaughan, A.M. (2014). Type II Fatty acid biosynthesis is essential for *Plasmodium falciparum* sporozoite development in the midgut of *Anopheles* mosquitoes. *Eukaryotic Cell*, *13*(5), 550–559.

Vanaerschot, M., Lucantoni, L., Li, T., Combrinck, J. M., Ruecker, A., Kumar, T. R. S., Rubiano, K., Ferreira, P. E., Siciliano, G., Gulati, S., Henrich, P. P., Ng, C. L., Murithi, J. M., Corey, V. C., Duffy, S., Lieberman, O. J., Veiga, M. I., Sinden, R. E., Alano, P., … Fidock, D. A. (2017). Hexahydroquinolines are antimalarial candidates with potent blood-stage and transmission-blocking activity. *Nature Microbiology*, *2*(10), 1403–1414.

Vennerstrom, J. L., Arbe-Barnes, S., Brun, R., Charman, S. A., Chiu, F. C. K., Chollet, J., Dong, Y., Dorn, A., Hunziker, D., & Matile, H. (2004). Identification of an antimalarial synthetic trioxolane drug development candidate. *Nature*, *430*(7002), 900–904.

Veverytsa, L., & Allan, D. W. (2013). Subtype-specific neuronal remodeling during *Drosophila* metamorphosis. *Fly*, *7*(2), 78–86.

Vinetz, J. M., Dave, S. K., Specht, C. A., Brameld, K. A., Xu, B., Hayward, R., & Fidock, D. A. (1999). The chitinase PfCHT1 from the human malaria parasite *Plasmodium falciparum* lacks proenzyme and chitin-binding domains and displays unique substrate preferences. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(24), 14061–14066.

Vizioli, J., Bulet, P., Hoffmann, J. A., Kafatos, F. C., Müller, H.-M., & Dimopoulos, G. (2001). Gambicin: A novel immune responsive antimicrobial peptide from the malaria vector *Anopheles gambiae. Proceedings of the National Academy of Sciences*, *98*(22), 12630 – 12635.

Vlachou, D., Schlegelmilch, T., Christophides, G. K., & Kafatos, F. C. (2005). Functional genomic analysis of midgut epithelial responses in *Anopheles* during *Plasmodium* invasion. *Current Biology*, *15*(13), 1185–1195.

Volohonsky, G., Hopp, A.-K., Saenger, M., Soichot, J., Scholze, H., Boch, J., Blandin, S. A., & Marois, E. (2017). Transgenic expression of the Anti-parasitic factor TEP1 in the malaria mosquito *Anopheles gambiae*. *PLoS Pathogens*, *13*(1), e1006113.

Vongvanich, N., Kittakoop, P., Charoenchai, P., Intamas, S., Sriklung, K., & Thebtaranonth, Y. (2006). Antiplasmodial, antimycobacterial, and cytotoxic principles from *Camchaya calcarea*. *Planta Med*, *72*(15), 1427–1430.

Wadi, I., Pillai, C. R., Anvikar, A. R., Sinha, A., Nath, M., & Valecha, N. (2018). Methylene blue induced morphological deformations in *Plasmodium falciparum* gametocytes: implications for transmission-blocking. *Malaria Journal*, *17*(1), 11.

Wahyuni, T. S., Ekasari, W., & Widyawaruyanti, A. (2009). Artopeden A, a new antiplasmodial isoprenylated flavone from *Artocarpus champeden*. *Heterocycles*, *79*(1), 1121–1126.

Waite, J. L., Swain, S., Lynch, P. A., Sharma, S. K., Haque, M. A., Montgomery, J., & Thomas, M. B. (2017). Increasing the potential for malaria elimination by targeting zoophilic vectors. *Scientific Reports*, *7*(1), 1–10.

Wang, C.-F., Yang, K., You, C.-X., Zhang, W.-J., Guo, S.-S., Geng, Z.-F., Du, S.-S., & Wang, Y.-Y. (2015). Chemical composition and insecticidal activity of essential oils from *Zanthoxylum dissitum* leaves and roots against three species of storage pests. *Molecules*, *20*(5), 7990–7999.

Warhurst, D. C., Craig, J. C., Adagu, I. S., Guy, R. K., Madrid, P. B., & Fivelman, Q. L. (2007). Activity of piperaquine and other 4-aminoquinoline antiplasmodial drugs against chloroquine-sensitive and resistant blood-stages of *Plasmodium falciparum*: Role of β-haematin inhibition and drug concentration in vacuolar water- and lipid-phases. *Biochemical Pharmacology*, *73*(12), 1910–1926.

Warncke, J. D., Vakonakis, I., & Beck, H.-P. (2016). *Plasmodium* Helical Interspersed Subtelomeric (PHIST) proteins, at the center of host cell remodeling. *Microbiology*

and Molecular Biology Reviews 80(4), 905–927.

Waters, A. P., & Janse, C. J. (2004). *Malaria parasites: genomes and molecular biology*. Horizon Scientific Press.

Wells, T. N. C. (2011). Natural products as starting points for future anti-malarial therapies: going back to our roots? *Malaria Journal*, *10*(1), S3.

Wells, T. N. C., Alonso, P. L., & Gutteridge, W. E. (2009). New medicines to improve control and contribute to the eradication of malaria. *Nature Reviews Drug Discovery*, *8*(11), 879–891.

White, B. H., & Ewer, J. (2014). Neural and hormonal control of postecdysial behaviors in insects. *Annual Review of Entomology*, *59*, 363–381.

White, M. W., & Suvorova, E. S. (2018). Apicomplexa cell cycles: Something old, borrowed, lost, and new. *Trends in Parasitology*, *34*(9), 759–771.

WHO. (1997). *Chemical methods for the control of vectors and pests of public health importance*. World Health Organization.

WHO. (2003). *Guidelines on good agricultural and collection practices (GACP) for medicinal plants.*

WHO. (2005). Guidelines for laboratory and field testing of mosquito larvicides. *World Health Organization*, https://doi.org/Ref: WHO/CDS/WHOPES/GCDPP/2005.11

WHO. (2009). *Global insecticide use for vector-borne disease control* (Fourth, Issue WHO/HTM/NTD/WHOPES/GCDPP/2009.6). World Health Organization.

Widyawaruyanti, A., Subehan, Kalauni, S. K., Awale, S., Nindatu, M., Zaini, N. C., Syafruddin, D., Asih, P. B. S., Tezuka, Y., & Kadota, S. (2007). New prenylated flavones from *Artocarpus champeden*, and their antimalarial activity in vitro. *Journal of Natural Medicines*, *61*(4), 410–413.

Wilkins, S., & Billingsley, P. F. (2001). Partial characterization of oligosaccharides expressed on midgut microvillar glycoproteins of the mosquito, *Anopheles stephensi* Liston. *Insect Biochemistry and Molecular Biology*, *31*(10), 937–948.

Willcox, M., Bodeker, G., Rasoanaivo, P., & Addae-Kyereme, J. (2004). *Traditional medicinal plants and malaria*. CRC press.

Wirth, C. C., Glushakova, S., Scheuermayer, M., Repnik, U., Garg, S., Schaack, D., Kachman, M. M., Weißbach, T., Zimmerberg, J., Dandekar, T., Griffiths, G., Chitnis, C. E., Singh, S., Fischer, R., & Pradel, G. (2014). Perforin-like protein PPLP2 permeabilizes the red blood cell membrane during egress of *Plasmodium falciparum* gametocytes. *Cellular Microbiology*, *16*(5), 709–733.

Wong, W., Bai, X.-C., Sleebs, B. E., Triglia, T., Brown, A., Thompson, J. K., Jackson, K. E., Hanssen, E., Marapana, D. S., & Fernandez, I. S. (2017). Mefloquine targets the *Plasmodium falciparum* 80S ribosome to inhibit protein synthesis. *Nature Microbiology*, *2*(6), 17031.

Wongsrichanalai, C., Pickard, A. L., Wernsdorfer, W. H., & Meshnick, S. R. (2002). Epidemiology of drug-resistant malaria. *The Lancet Infectious Diseases*, *2*(4), 209–218.

Wright, C. W. (2007). Recent developments in naturally derived antimalarials: cryptolepine analogues. *Journal of Pharmacy and Pharmacology*, *59*(6), 899–904.

Wu, J.-M., Chen, Y., Chen, J.-C., Lin, T.-Y., & Tseng, S.-H. (2010). Tetrandrine induces apoptosis and growth suppression of colon cancer cells in mice. *Cancer Letters*, *287*(2), 187–195.

Wu, S.-J., & Ng, L.-T. (2007). Tetrandrine inhibits proinflammatory cytokines, iNOS and COX-2 expression in human monocytic cells. *Biological and Pharmaceutical Bulletin*, *30*(1), 59–62.

Wu, T., Nagle, A., Kuhen, K., Gagaring, K., Borboa, R., Francek, C., Chen, Z., Plouffe, D., Goh, A., & Lakshminarayana, S. B. (2011). Imidazolopiperazines: hit to lead optimization of new antimalarial agents. *Journal of Medicinal Chemistry*, *54*(14), 5116–5130.

Yakubu, R. R., Weiss, L. M., & Silmon de Monerri, N. C. (2018). Post-translational modifications as key regulators of apicomplexan biology: insights from proteome-wide studies. *Molecular Microbiology*, *107*(1), 1–23.

Yang, C., & Arrizabalaga, G. (2017). The serine/threonine phosphatases of apicomplexan parasites. *Molecular Microbiology*, *106*(1), 1–21.

Yang, T., Ottilie, S., Istvan, E. S., Godinez-Macias, K. P., Lukens, A. K., Baragaña, B., Campo, B., Walpole, C., Niles, J. C., & Chibale, K. (2021). MalDA, accelerating malaria drug discovery. *Trends in Parasitology*, *37*(6), 493–507.

Yanow, S. K., Purcell, L. A., Pradel, G., Sato, A., Rodriguez, A., Lee, M., & Spithill, T. W. (2008). Potent antimalarial and transmission-blocking activities of Centanamycin, a novel DNA-binding agent. *The Journal of Infectious Diseases*, *197*(4), 527–534.

Ye, X., Yang, L., Stanley, D., Li, F., & Fang, Q. (2017). Two *Bombyx mori* acetylcholinesterase genes influence motor control and development in different ways. *Scientific Reports*, *7*(1), 4985.

Yerbanga, R. S., Lucantoni, L., Ouédraogo, R. K., Da, D. F., Yao, F. A., Yaméogo, K. B., Churcher, T. S., Lupidi, G., Taglialatela-Scafati, O., & Gouagna, L. C. (2014). Transmission blocking activity of *Azadirachta indica* and *Guiera senegalensis* extracts on the sporogonic development of *Plasmodium falciparum* field isolates in *Anopheles coluzzii* mosquitoes. *Parasites & Vectors*, *7*(1), 185.

Yuda, M., Iwanaga, S., Kaneko, I., & Kato, T. (2015). Global transcriptional repression: An initial and essential step for *Plasmodium* sexual development. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(41), 12824–12829.

Yuda, M., Iwanaga, S., Shigenobu, S., Mair, G. R., Janse, C. J., Waters, A. P., Kato, T., & Kaneko, I. (2009). Identification of a transcription factor in the mosquito-invasive stage of malaria parasites. *Molecular Microbiology*, *71*(6), 1402–1414.

Zhang, B., Watts, K. M., Hodge, D., Kemp, L. M., Hunstad, D. A., Hicks, L. M., & Odom, A. R. (2011). A second target of the antimalarial and antibacterial agent fosmidomycin revealed by cellular metabolic profiling. *Biochemistry*, *50*(17), 3570–3577.

Zhang, L., Lu, Y., Xiang, M., Shang, Q., & Gao, X. (2016). The retardant effect of 2-tridecanone, mediated by cytochrome P450, on the development of Cotton bollworm, *Helicoverpa armigera*. *BMC Genomics*, *17*(1), 954.

Zhang, M., Fennell, C., Ranford-Cartwright, L., Sakthivel, R., Gueirard, P., Meister, S., Caspi, A., Doerig, C., Nussenzweig, R. S., & Tuteja, R. (2010). The *Plasmodium* eukaryotic initiation factor-2α kinase IK2 controls the latency of sporozoites in the mosquito salivary glands. *Journal of Experimental Medicine*, *207*(7), 1465–1474.

Zhang, X., Wang, X., Wu, T., Li, B., Liu, T., Wang, R., Liu, Q., Liu, Z., Gong, Y., & Shao, C. (2015). Isoliensinine induces apoptosis in triple-negative human breast cancer cells through ROS generation and p38 MAPK/JNK activation. *Scientific Reports*, *5*(1), 12579.

Zhang, Y., Peng, Y., Chen, F., Zeng, D., Huang, Y. I., & Zhong, H. (2009). Bioactivity and components of essential oil from *Zanthoxylum ailanthoides* fruits against two mosquito species. *Acta Entomologica Sinica*, *52*(9), 1028–1033.

Zhao, L.-X., Li, D.-D., Hu, D.-D., Hu, G.-H., Yan, L., Wang, Y., & Jiang, Y.-Y. (2013). Effect of tetrandrine against *Candida albicans* biofilms. *PloS One*, *8*(11), e79671–e79671.

Zieler, H., Keister, D. B., Dvorak, J. A., & Ribeiro, J. M. C. (2001). A snake venom phospholipase A2 blocks malaria parasite development in the mosquito midgut by inhibiting ookinete association with the midgut surface. *Journal of Experimental Biology*, *204*(23), 4157–4167.

Zieler, H., Nawrocki, J. P., & Shahabuddin, M. (1999). *Plasmodium gallinaceum* ookinetes adhere specifically to the midgut epithelium of *Aedes aegypti* by interaction with a carbohydrate ligand. *Journal of Experimental Biology*, *202(5)*, 485–495.

Zuzarte-Luís, V., & Mota, M. M. (2018). Parasite sensing of host nutrients and environmental cues. *Cell Host & Microbe*, *23*(6), 749–758.

APPENDICES

Appendix I: Predicted isoliensinine (CHEMBL502370) protein targets.

Serial	ChEMBL ID[a]	Eukaryotic target name	*Plasmodium* homologue ID[b]	Protein name on PlasmoDB	Protein length (aa)	Cellular function in *Plasmodium*
1	CHEMBL4358	Arachidonate 15-lipoxygenase	Pf3D7_ 0419600	Ran-specific GTPase-activating protein 1, putative	280	Intracellular transport
2	CHEMBL312	Arachidonate 5-lipoxygenase	Pf3D7_1321500	3',5'-cyclic nucleotide phosphodiesterase beta, unspecified product	1139	Signal transduction
3	CHEMBL4244	Legumain	-	Unknown	-	Unknown
4	CHEMBL4070	Casein kinase II alpha	Pf3D7_1108400	Casein kinase 2, alpha subunit	335	Peptidyl-serine phosphorylation
5	CHEMBL2096675	Integrin α-V/β5	Pf3D7_1475400	Cysteine repeat modular protein 4	5906	Ligand/receptor binding
6	CHEMBL3024	Serine/threonine protein kinase PLK1	Pf3D7_1246900	RAC-beta serine/threonine protein kinase	735	Mitotic spindle assembly
7	CHEMBL4161	Urease	Pf3D7_0603200	Mitochondrial chaperone BCS1, putative	471	Stress response
8	CHEMBL4777	Neuropeptide Y receptor type 1	Pf3D7_0208100	Conserved Plasmodium protein, unknown function	1789	Unknown
9	CHEMBL276	Muscarinic acetylcholine receptor M1	Pf3D7_1319700	Protein phosphatase PP2A regulatory subunit A, putative	858	Gametocyte-specific growth
10	CHEMBL5023	p53-binding protein mdm-2	Pf3D7_0518200	SWIB/MDM2 domain-containing protein	131	Stress response
11	CHEMBL2411	Serotonin 3a (5HT3a) receptor	Pf3D7_0710000	Conserved Plasmodium protein, unknown function	3267	Unknown
12	CHEMBL4123	Neurotensin receptor 1	-	Unknown	-	Unknown
13	CHEMBL326	Alpha-1d adrenergic receptor	Pf3D7_0723700	metallo-hydrolase/oxidoreductase, putative	1032	tRNA modifications
14	CHEMBL278	Integrin alpha 4	-	Unknown	-	Unknown

15	CHEMBL5451	Sodium channel protein type X alpha subunit	-	Unknown	-	Unknown
16	CHEMBL4766	Pyruvate dehydrogenase kinase isoform 1	-	Unknown	-	Unknown
17	CHEMBL5568	Proto-oncogene tyrosine protein kinase ROS	Pf3D7_0211700	Tyrosine kinase-like protein, putative	1233	Protein phosphorylation
18	CHEMBL1671613	Type 1 angiotensin II receptor	Pf3D7_1235200	V-type K+-independent H+-translocating inorganic pyrophosphatase	1057	Ion homeostasis
19	CHEMBL5285	Mitogen activated protein kinase kinase 5	Pf3D7_0610600	Calcium-dependent protein kinase 2	509	Male exflagellation
20	CHEMBL4398	Purinergic receptor P2Y2	Pf3D7_0812900	Conserved Plasmodium protein, unknown function	1567	Unknown
21	CHEMBL3137262	LSD1/CoREST complex	-	Unknown	-	Unknown
22	CHEMBL3975	Fructose-1,6-bisphosphatase	-	Unknown	-	Unknown
23	CHEMBL5113	Orexin receptor 1	-	Unknown	-	Unknown
24	CHEMBL4608	Melanocortin receptor 5	Pf3D7_1021500	ATP-dependent RNA helicase ROK1, putative	680	Pre-mRNA splicing
25	CHEMBL5747	CREB binding protein	Pf3D7_1212900	Bromodomain protein 2, putative	1088	
26	CHEMBL1795139	Transmembrane protease serine 6	PF3D7_1362900	Conserved Plasmodium protein, unknown function	1636	Unknown
27	CHEMBL2781	Sodium/hydrogen exchanger 1	Pf3D7_1303500	Sodium/hydrogen exchanger	1920	Cytosolic pH regulation and quinine resistance
28	CHEMBL5141	CYP26A1	-	Unknown	-	Unknown
29	CHEMBL5763	Cholinesterase	Pf3D7_0302500	Cytoadherence linked asexual protein 3.1	1417	Cytoadherence
30	CHEMBL1947	Thyroid hormone receptor beta 1	Pf3D7_1123100	Calcium-dependent protein kinase 7	2265	Cyclic nucleotide signaling
31	CHEMBL3774295	Lysine-specific	Pf3D7_0809900	JmjC domain-containing	1259	Histone

		demethylase 5B		protein, putative		modification
32	CHEMBL4791	Endothelin converting enzyme 1	-	Unknown	-	Unknown
33	CHEMBL5845	Glycine receptor subunit 1α	-	Unknown	-	Unknown
34	CHEMBL235	Perixosome proliferation-activated receptor gamma	Pf3D7_0712800	Erythrocyte membrane protein 1, PfEMP1	2239	RBC invasion
35	CHEMBL1287623	Lethal (3) malignant brain tumor like protein 3	PfGA01_130031900	Conserved Plasmodium protein, unknown function	-	Unknown
36	CHEMBL3816	Cytosolic phospholipase A2	Pf3D7_1038800	RESA-like protein with PHIST and DnaJ domains	911	RBC surface remodelling
37	CHEMBL3976	Dipeptidyl peptidase II	Pf3D7_1247800	Dipeptidyl aminopeptidase 2	590	Proteolysis
38	CHEMBL3037	Cannabinoid CB1 receptor	-	Unknown	-	Unknown
39	CHEMBL1907603	Glutamate NMDA receptor; GRIN1/GRIN2B	Pf3D7_0710200	Conserved Plasmodium protein, unknown function	2910	Unknown
40	CHEMBL2575	Dihydrofolate reductase	Pf3D7_0417200	Bifunctional dihydrofolate reductase-thymidylate synthase	608	Folate metabolism
41	CHEMBL1849	Enoyl-[acyl carrier protein] reductase	Pf3D7_0615100	Enoyl-acyl carrier reductase	432	Fatty acid biosynthesis
42	CHEMBL2096912	Protein farnesyltransferase	Pf3D7_1242600	Protein farnesyltransferase subunit alpha	521	Protein farnesylation
43	CHEMBL1908385	Serine/threonine protein kinase pknB	Pf3D7_0610600	Calcium-dependent protein kinase 2	509	Male gamete exflagellation
44	CHEMBL288	Phosphodiesterase 4D	Pf3D7_1321500	3',5'-cyclic nucleotide phosphodiesterase beta, unspecified product	1139	Signal transduction
45	CHEMBL1860	Thyroid hormone receptor alpha	Pf3D7_1474100	Conserved Plasmodium protein, unknown function	121	Unknown
46	CHEMBL1940	Voltage-gated L-type calcium channel alpha	Pf3D7_0830500	Sporozoite and liver stage tryptophan-rich protein,	675	Putative amino acid transporter

		1C subunit		putative		
47	CHEMBL2016	Coagulation factor IX	Pf3D7_0323400	Rh5 interacting protein	1086	Rosetting
48	CHEMBL3105	Poly (ADP-ribose) polymerase 1	Pf3D7_1412100	Meiosis-specific nuclear structural protein 1, putative	956	Cell cycle regulations
49	CHEMBL3667	PI4-kinase α-subunit	Pf3D7_0509800	Phosphatidylinositol 4-kinase	1559	Inositol Phosphate metabolism
50	CHEMBL1856	Steroid 5-alpha reductase 2	Pf3D7_ 1135900	3-oxo-5-alpha-steroid 4-dehydrogenase, putative	296	Glycerophospholipid synthesis
51	CHEMBL1741186	Nuclear receptor ROR-gamma	-	Unknown	-	Unknown
52	CHEMBL3891	Calpain 1	Pf3D7_1362400	Calpain	2048	Nucleolar protein trafficking
53	CHEMBL3359	Formyl peptide receptor 1	-	Unknown	-	Unknown
54	CHEMBL5983	Aldo-keto reductase family 1 membrane B10	Pf3D7_1364600	Aldehyde reductase, putative	880	Glyoxalase metabolism
55	CHEMBL1892	Glutamate carboxypeptidase II	Pf3D7_1200600	Erythrocyte membrane protein 1, PfEMP1	3056	RBC invasion

[a]Predictions performed at 70-90% confidence and only targets labelled “Active” selected.

[b]Homology search based on *BLASTp* of target sequences in PlasmoDB. (-) denotes lack of homology in *Plasmodium* annotations.

Appendix II: Predicted protein targets of Juliprosopine (Compound 87; CHEMBL540975).

Serial	ChEMBL ID[a]	Eukaryotic target name	*Plasmodium* homologue ID[b]	Protein name on PlasmoDB	Protein length	Cellular function in *Plasmodium*
1	CHEMBL5062	Coagulation factor X	Pf3D7_0206900	Merozoite surface protein 5	261	RBC invasion
2	CHEMBL2016	Coagulation factor IX	PBANKA_ 1215100	Rh5 interacting protein, putative	1096	RBC invasion
3	CHEMBL3774295	Lysine-specific demethylase 5B	PF3D7_0809900	JmjC domain-containing protein, putative	1259	Histone demethylation
4	CHEMBL1835	Thromboxane A synthase	PBANKA_ 0909800	RNA-binding protein, putative	1480	RNA binding
5	CHEMBL1821	Muscarinic acetyl receptor M4	Pf TG01_000063400	Erythrocyte membrane protein 1, PfEMP1	1289	RBC invasion
6	CHEMBL211	Muscarinic acetyl receptor M2	Pf3D7_ 1321100	Conserved Plasmodium protein, unknown function	3447	Unknown
7	CHEMBL2035	Muscarinic acetyl receptor M5	Pf3D7_ 1321100	Conserved Plasmodium protein, unknown function	3447	Unknown
8	CHEMBL340	CYP3A4	Pf3D7_ 1205700	Targeted glyoxalase II	322	GSH metabolism in detoxification
9	CHEMBL5845	Glycine receptor subunit alpha 1	-	Unknown	-	-
10	CHEMBL3199	Acetylcholinesterase	-	Unknown	-	-
11	CHEMBL1914	Butyryl cholinesterase	PfTG01_040032900	Erythrocyte membrane protein 1, PfEMP1	2193	RBC invasion
12	CHEMBL2830	Voltage gated L-type calcium channel 1-C alpha subunit	-	Unknown	-	-
13	CHEMBL1942	Alpha-2b adrenergic receptor	PfML01_040018200	Erythrocyte membrane protein 1, PfEMP1	2244	RBC invasion
14	CHEMBL3589	Adenosine kinase	PVP01_1331500	Rap guanine nucleotide exchange factor, putative	3356	Nucleotide binding
15	CHEMBL1287623	Lethal (3) malignant brain tumor-like protein 3	PfGA01_130031900	Conserved Plasmodium protein, unknown function	1534	Unknown

16	CHEMBL3922	Methionine aminopeptidase 2	Pf3D7_1434600	Methionine aminopeptidase 2	628	Cellular protein modification
17	CHEMBL5375	HCV NS5B RNA-dependent RNA polymerase	-	Unknown	-	-
18	CHEMBL1856	Steroid 5-alpha reductase 2	Pf3D7_1135900	3-oxo-5-alpha-steroid 4-dehydrogenase, putative	296	Fatty acid biosynthesis
19	CHEMBL4244	Legumain	Pf3D7_1128700	GPI-anchor transamidase, putative	493	Proteolysis
20	CHEMBL2096675	Integrin alpha-V/Beta 5	Pf3D7_1475400	Cysteine repeat modular protein 4	5906	mRNA processing by ubiquitinylation
21	CHEMBL3105	Poly(ADP-ribose) polymerase 1	PVP01_1412100	Meiosis-specific nuclear structural protein 1, putative	495	Gametocytogeenesis
22	CHEMBL1795139	Transmembrane protease serine 6	Pf3D7_1362900	Conserved Plasmodium protein, unknown function	1636	Unknown
23	CHEMBL2637	c-Jun N-terminal kinase 3	Pf3D7_1431500	Mitogen-activated protein kinase 1	914	Protein phosphorylation
24	CHEMBL335	Protein tyrosine phosphatase B	PADL01_0514300	Phosphatidylinositol 3-kinase	2074	Phosphatidylinositol phosphorylation
25	CHEMBL3359	Formyl peptide receptor 1	-	Unknown	-	-
26	CHEMBL5983	Aldo-keto reductase family 1 member B10	PF3D7_1364600	Aldehyde reductase, putative	880	Cellular metabolism
27	CHEMBL286	Renin	Pf3D7_1407900	Plasmepsin I	452	Heme metabolism
28	CHEMBL4860	Apoptosis regulator Bcl-2	PVP 01_0417700	Serine-repeat antigen (SERA)	1127	RBC egress
29	CHEMBL4644	Melanocortin receptor 3	PVP01_0006160	Plasmodium exported protein, unknown function	251	Unknown
30	CHEMBL267	Tyrosine protein kinase SRC	Pf3D7_1315100	Serine/threonine protein kinase PK9	367	Histone phosphorylation
31	CHEMBL4062	Calpain 1	PBANKA_1138400	Calpain, putative	1922	Cell cycle progression

32	CHEMBL2094108	Protein farnesyl transferase	PF3D7_1242600	Protein farnesyltransferase subunit alpha	521	Protein farnesylation
33	CHEMBL1908385	Serine/threonine protein kinase pknB	Pf3D7_0610600	Calcium-dependent protein kinase 2	513	Male gamete exflagellation
34	CHEMBL288	Phosphodiesterase 4D	Pf3D7_1321500	3',5'-cyclic nucleotide phosphodiesterase beta, unspecified product	1139	Signal transduction
35	CHEMBL275	Phosphodiesterase 4B	Pf3D7_1321500	3',5'-cyclic nucleotide phosphodiesterase beta, unspecified product	1139	Signal transduction
36	CHEMBL1860	Thyroid hormone receptor alpha	Pf3D7_1474400	Conserved Plasmodium protein, unknown function	2977	-
37	CHEMBL3910	11-beta hydroxysteroid dehydrogenase 1	Pf3D7_0922900	3-oxoacyl-[acyl-carrier-protein] reductase	301	Fatty acid biosynthesis
38	CHEMBL1849	Enoyl[acyl carrier protein]reductase	Pf3D7_0615100	Enoyl-acyl carrier reductase	432	Fatty acid biosynthesis
39	CHEMBL3712907	Transmembrane domain-containing protein TMIGD3	-	Unknown	-	-
40	CHEMBL3037	Cannabinoid CB1 receptor	-	Unknown	-	-
41	CHEMBL3976	Dipeptidyl peptidase II	-	Unknown	-	-
42	CHEMBL4766	Pyruvate dehydogenase kinase isoform 1	PF3D7_1128500	Fe-S cluster assembly protein, putative	718	Iron-sulfur cluster assembly
43	CHEMBL4203	Dual specificity protein kinase CLK4	Pf3D7_1445400	Protein serine/threonine kinase-1 (CLK1)	881	Regulation of cell differentiation
44	CHEMBL2329	Dihydrofolate reductase	Pf3D7_0417200	Bifunctional dihydrofolate reductase-thymidylate synthase	608	DNA synthesis
45	CHEMBL4123	Neurotensin receptor 1	-	Unknown	-	-
46	CHEMBL5451	Sodium channel protein type X alpha subunit	-	Unknown	-	-
47	CHEMBL4653	Dipeptidyl peptidase IV	Pf3D7_0321500	Peptidase,	1074	Methionine

				putative		removal from proteins
48	CHEMBL4051	Cystic fibrosis transmembrane conductance regulator	Pf3D7_1352100	ABC transporter B family member 6, putative	1049	Transmembrane transport
49	CHEMBL3892	Sphingosine1-phosphate receptor EDG-3	-	Unknown	-	-
50	CHEMBL2304404	Adenosine α-1 receptor	Pf3D7_0826100	HECT-like E3 ubiquitin ligase, putative	7263	Protein ubiquitination
51	CHEMBL3242	Carbonic anhydrase XII	Pf3D7_1140000	Carbonic anhydrase	600	Carbonate dehydratase activity
52	CHEMBL2283	Carbonic anhydrase II	Pf3D7_1140000	Carbonic anhydrase	600	Carbonate dehydratase activity
53	CHEMBL3524	Histone deacetylase 4	PF3D7_1008000	Histone deacetylase 2	2379	Histone modification
54	CHEMBL5141	CYP26A1	-	Unknown	-	-
55	CHEMBL4878	CYP1B1	Pf3D7_1428800	Transcription initiation TFIID-like, putative	367	RNA metabolism
56	CHEMBL5747	CREB-binding protein	Pf3D7_1212900	Bromodomain protein 2, putative	1088	Chromatin remodelling
57	CHEMBL2002	Inosine-5-monophosphate dehydrogenase 2	Pf3D7_0920800	Inosine 5'-monophosphate dehydrogenase	510	DNA Synthesis
58	CHEMBL3975	Fructose1,6-bisphosphatase	-	Unknown	-	-
59	CHEMBL4791	Endothelin-converting enzyme 1	-	Unknown	-	-
60	CHEMBL2919	Glutamate receptor ionotropic kainate 1	Pf3D7_0400800	Stevor	289	Virulence
61	CHEMBL5285	Mitogen-activated protein kinase kinase 5	Pf3D7_0610600	Calcium-dependent protein kinase 2	513	Male gamete exflagellation
62	CHEMBL3137262	LSD-1/COREST complex	-	Unknown	-	-
63	CHEMBL3667	PI4K kinase alpha subunit	Pf3D7_0509800	Phosphatidylinositol 4-kinase	1559	Phosphatidylinositol

						phosphorylation
64	CHEMBL4336	Prostanoid EP3 receptor	-	Unknown	-	-
65	CHEMBL1741186	Nuclear receptor ROR-gamma	-	Unknown	-	-
66	CHEMBL3374	Angiotensin II type 1a (AT-1a) receptor	-	Unknown	-	-

[a]Predictions performed at 70-90% confidence and only targets labelled "Active" selected.

[b]Homology search based on *BLASTp* of target sequences in PlasmoDB. (-) denotes lack of homology in *Plasmodium* annotation

Appendix III: *In silico* prediction of drug-likeness properties for Isoliensinine (254) and Juliprosopine (87). SwissADME was used to predict all the correspondent values presented.

Property	**Isoliensinine**	**Juliprosopine**
MW	610.7	630.04
Consensus lipophilicity (Log $P_{o/w}$)	4.87	7.96
Water solubility	Poor	Poor
TPSA	83.86	67.76
P-gp inhibitor	Yes	-
P-gp substrate	Yes	No
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	Yes	No
CYP3A4 inhibitor	No	Yes
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
GI absorption	High	Low
Bioavailability score	0.55	0.17
Lipinsiki drug-likeness	Yes; 1 violation: MW>500	2 violations: Mw>500; Mlog P > 4.15
PAINS	No	No

Appendix IV: Larvicidal activity screening of ZCE fractions against *An. gambiae* larvae.

Fraction	Concentration (ppm)	% mean larval mortality[a]
ZCFr.1	1	0 ± 0.00
(100% hexane)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.2	1	0 ± 0.00
(9:1 hexane/EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.3	1	0 ± 0.00
(4:1 hexane/EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.4	1	0 ± 0.00
(7:3 hexane/EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	4 ± 1.20
ZCFr.5	1	20 ± 2.12
(3:2 hexane/EtAOc)	2.5	72 ± 0.00
	5	84 ± 1.78
	7.5	88 ± 0.24
	10	100 ± 0.00
ZCFr.6	1	0 ± 0.00
(1:1 hexane/EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.7	1	0 ± 0.00
(2:3 hexane/EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.8	1	0 ± 0.00
(3:7 hexane/EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.9	1	0 ± 0.00
(1:4 hexane/EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00

	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.10	1	0 ± 0.00
(1:9 hexane/EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.11	1	0 ± 0.00
(100% EtAOc)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00
ZCFr.12	1	0 ± 0.00
(100% MeoH)	2.5	0 ± 0.00
	5	0 ± 0.00
	7.5	0 ± 0.00
	10	0 ± 0.00

[a]Assay conducted twice (n = 2 replicates)

Appendix V: GC-MS analysis results of ZCFr.5.

GC-MS analysis results of ZCFr.5 providing retention time (RT), the compound name, peak area and respective area percent of the total spectrum that denotes abundance.

RT	Compound name	Area	Area %
14.7225	Dodecane, 4,6-dimethyl-	434246	0.004
15.4421	19.90 Undecanone<2->	66861808	0.662
15.5415	2-Undecanol	4634539	0.046
16.2669	cis-muurola-3,5-diene	982575	0.010
16.8168	Methyl decyl ketone	7556206	0.075
17.0157	Vanillin	12347878	0.122
17.2731	b- Cedrene	2809024	0.028
17.6417	2-Tridecanone	2625662	0.026
17.8698	Cyclodecane	13990732	0.139
17.9751	1,5-Cyclodecadiene, (E,Z)-	7145894	0.071
18.2267	Tridecanone<2->	1092622128	10.822
18.332	Phenol, 2,4-bis(1,1-dimethylethyl)-	16161950	0.160
18.9462	Nerolidol<E->	42438158	0.420
19.3148	2-Tetradecanone	7919472	0.078
19.5371	Cedrol	84040782	0.832
19.7594	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	9327897	0.092
19.9817	Muurolol<alpha-> (=Torreyol)	7385935	0.073
20.3912	Dodecyl acrylate	68540245	0.679
21.1809	Tetradecanoic acid	88380521	0.875
21.959	Cyclopentadecanone, 2-hydroxy-	5250242	0.052
22.0116	9-Tetradecenal, (Z)-	5410326	0.054
22.0467	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	25937327	0.257
22.1696	Pentadecanoic acid	19006569	0.188
22.4563	Methyl hexadecanoate	2569965	0.025
22.7663	Benzenepropanoic acid, 4-hydroxy-, methyl ester	13651562	0.135
22.8248	Methyl palmitate (Methyl hexadecanoate)	56005160	0.555
23.0354	cis-9-Hexadecenoic acid	47547854	0.471
23.3864	Hexadecanoic acid	778071722	7.706
23.7023	Propanoic acid, 3-mercapto-, dodecyl ester	20901947	0.207
23.9773	E-9-Tetradecenoic acid	32296441	0.320
24.3166	Isoprenyl cinnamate<(Z)-methyl->	138241443	1.369
24.457	Methyl linoleate	38679025	0.383

24.5155	9-Octadecenoic acid, methyl ester, (E)-	63276761	0.627
24.7378	Methyl octadecanoate	13963236	0.138
25.0829	9,12-Octadecadienoic acid (Z,Z)-	1124544071	11.138
25.1414	6-Octadecenoic acid	546515450	5.413
25.1824	9-Octadecenoic acid, (E)-	292837487	2.900
25.2643	Octadecanoic acid	176584887	1.749
25.8083	4(3H)-Pyrimidinone, 2-amino-5-[2-(3,5-dimethylphenoxy)ethyl]-6-methyl-	65715481	0.651
27.3469	2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester	99438290	0.985
27.5867	3-Phenyl-1,3-pentanediol	31733260	0.314
28.0313	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	15410066	0.153
28.1074	Furo[2,3-b]quinolin-4(9H)-one, 7,8-dimethoxy-9-methyl-	62361081	0.618
28.5871	2-Methyl-3-undecyl-1H-quinolin-4-one	29020728	0.287
29.371	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	72520495	0.718
29.605	Tetracosanoic acid, methyl ester	35506552	0.352
29.9034	Tetracosanoic acid	26325202	0.261
30.816	Benzo[a]naphthacene-8,13-dione	16795447	0.166
31.3015	Hexacosanoic acid, methyl ester	25371893	0.251
32.1498	3-Methyl-2-nonyl-1H-quinolin-4-one	57037317	0.565
32.3428	Benzoic acid, 4-(dimethylamino)-, methyl ester	20988136	0.208
33.7117	4-(2,4,5-Trimethoxy-phenyl)-3,4-dihydro-1H-benzo[h]quinolin-2-one	40455105	0.401
34.3786	1,3-Benzodioxole, 5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-	1153823008	11.428
34.6009	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane	240478164	2.382
35.028	[1,3]Benzodioxolo[5,6-c]phenanthridine, 12,13-dihydro-2,3-dimethoxy-12-methyl-	212561822	2.105
35.1742	3-Methyl-2-undecyl-1H-quinolin-4-one	90200594	0.893
35.8236	Stigmasterol	142015722	1.407
36.2857	Benzo[vwx]hexaphene	36485691	0.361
36.7713	.gamma.-Sitosterol	187063793	1.853
37.0463	Lanosterol	50875185	0.504
37.3036	.beta.-Amyrin	144775183	1.434
38.38	.beta.-Humulene	1048319784	10.383
38.4561	Lupeol	187092931	1.853
38.5263	Taraxasterol	482034657	4.774
38.5438	5.alpha.-Androstan-17.beta.-ol, 2.alpha.,3.alpha.-epoxy-3-methyl-	564369403	5.590

40.5387	4-Amino-7-methoxy-2-(p-tolyl)-5H-(1)benzopyrano(4,3-d)pyrimidin-5-one	16862302	0.167
43.4696	Propanoic acid, 3,3'-thiobis-, didodecyl ester	3523829	0.035
		10096658248	100.000

Appendix VI: Correlation coefficients of ZCE AChE inhibition to LC_{50}

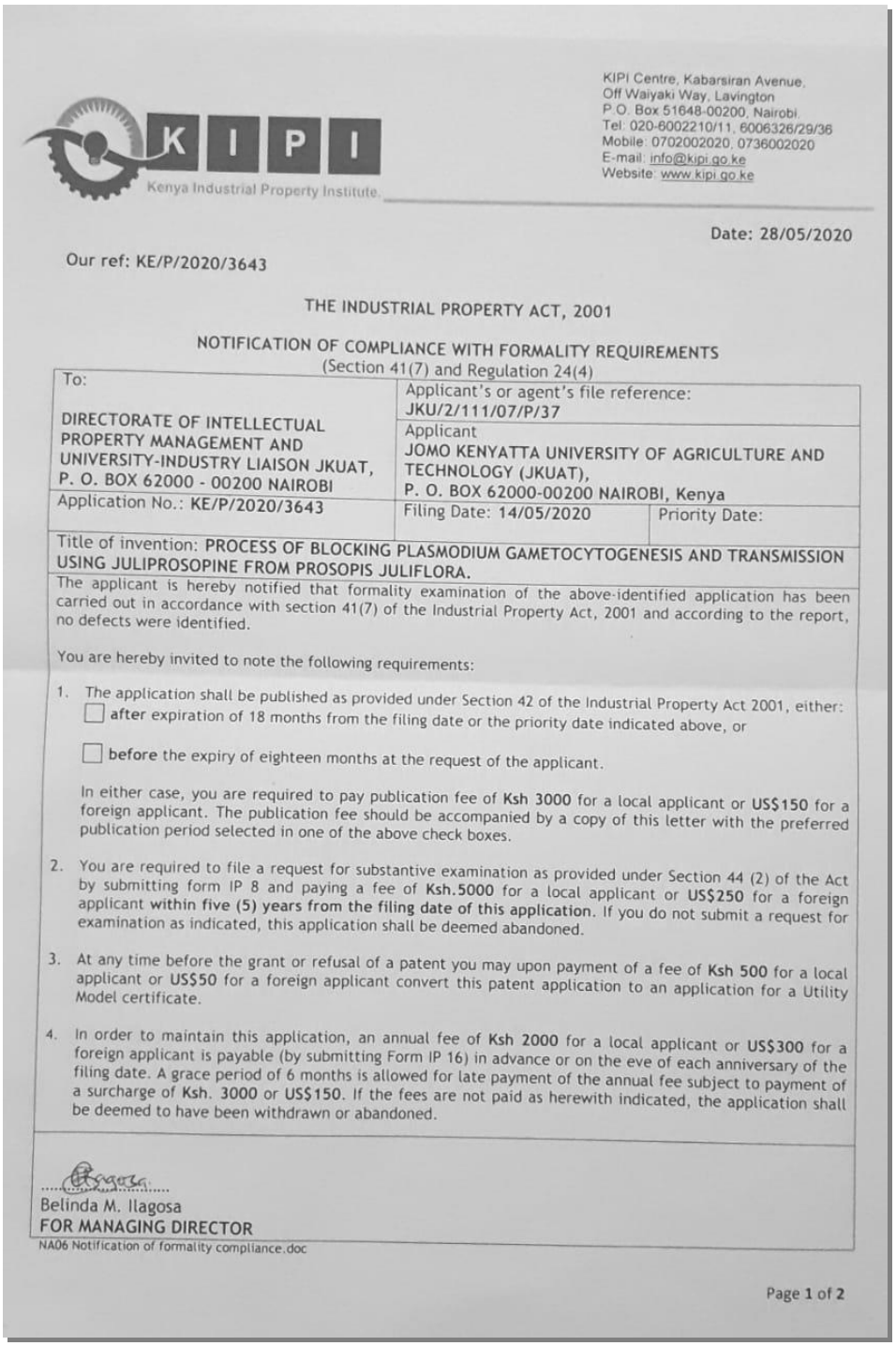
Mosquito species	**Correlation coefficient (*r*)**	r^2	***p* value**
An. gambiae	-0.8193	0.6713	0.0897
Ae. aegypti	-0.5844	0.3415	0.3007

Appendix VII: List of mosquito primers used for RT-qPCR.

Gene	Primer sequences (5'-3')[a]		PCR amplicon size (bp)
	Forward	Reverse	
AGAP010592 _Rsp7	AGAAGAAGTTCAGCGGCAAG	GATGGTGGTCTGCTGGTTCT	248
AAEL004175 _Rsp7	CATGGACTTCCACACCAACA	GCTTCAGCATCTCCTTGGTC	248
AGAP001356 _Ace	CTACACCGAGGACGAGAAGG	AGCTGGGGCAGGTACTTCTT	228
AAEL000511_Ace	GAGCTGAACCCCTACGTGAA	GTACACGTTGTTGCCCTCCT	186
AGAP028634 _EcR	AGAAGAAGGCCCAGAAGGAG	GTAGATCACGGCCATCTGGT	224
AAEL009600_EcR	AGAAGAAGGCCCAGAAGGAG	GTAGATCACGGCCATCTGGT	212
AGAP012223_E75A	AGGAGTACGAGCAGGAGCTG	CACAGGTCGTTGATCAGGTG	241
AAEL007397_E75A	AGCTACCAGCAGAGCGTGAT	CTCCAGCACGATGTTCTTCA	240
AGAP009399_βFTZ-F1	CTGCTACAGGAGCATCAGCA	TTCTCGCTCTCCTTCAGCTC	222
AAEL001304_βFTZ-F1	GAGTACGTGGCCATGAAGGT	TGATGGTCAGCATCTCCTTG	220
***AGAP005992_CYP302A1* (*disembodied*)**	CCCAGGAGTTCATGGAGAAG	GTGGTGTAGCTGGTGGTGTG	211
***AAEL015655_CYP302A1* (*disembodied*)**	CTGCAGGCAGGAGAGGTACT	CAGCTTGGTCTCGATGTTCA	235
***AGAP 004665_CYP306A1* (*phantom*)**	CTGCAGGACATCGACAACAT	AGTTGATGGTCCTCCTGGTG	247
***AAEL004888_CYP306A1* (*phantom*)**	CTGAGGTGGCTGATCCTGTT	TGGTGTTCTTGCTCACCTTG	235
***AGAP001039_CYP307A1* (*spook*)**	CAGCGTGAGGTTCGACTACA	GTCTGCTCCCTCTCGTTCAC	210
AAEL009762 _CYP307A1 (spook)	TGAAGGTGCTGTACGAGTGG	TGTACACGTCGCCGTACTTC	246
***AGAP002429_CYP314A1* (*shade*)**	CCTGCATCCAGGAGAGCTAC	TTGTTCACCACGTTGTCGTT	223
***AAEL010946_CYP314A1* (*shade*)**	CCAGAACGAGCAGAACTTCC	CTGGCTGTGGTACTCCACCT	235
AGAP002231_dATAC HAT	TGCGAGTTCTGCCTGAAGTA	CTCACGGTCAGCACGTAGA A	245
AAEL000452_dATAC HAT	TGGACTACTGCCCCAACTTC	ACCTTCTTGGTGTCGGTGTC	187
AAEL006280_JHAMT	TGTTCTTCGAGACCCTGGAC	TCAGCTGGTCGTAGATGTCG	213
AGAP005256_JHAMT	GAAGGGCTGGACAATTTGAA	TTCTCTTCGCTGCCAGTTTT	146

[a]Gene-specific sequences retrieved from vectorbase.org and primers designed using primer3 (URL: https://bioinfo.ut.ee/primer3-0.4.0/) tool.

Appendix VIII: Patent registration and publication



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Kenya Industrial Property Institute.

Date: 28/05/2020

Our ref: KE/P/2020/3643

THE INDUSTRIAL PROPERTY ACT, 2001

NOTIFICATION OF COMPLIANCE WITH FORMALITY REQUIREMENTS
(Section 41(7) and Regulation 24(4)

To:	Applicant's or agent's file reference: JKU/2/111/07/P/37	
DIRECTORATE OF INTELLECTUAL PROPERTY MANAGEMENT AND UNIVERSITY-INDUSTRY LIAISON JKUAT, P. O. BOX 62000 - 00200 NAIROBI	Applicant JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY (JKUAT), P. O. BOX 62000-00200 NAIROBI, Kenya	
Application No.: KE/P/2020/3643	Filing Date: 14/05/2020	Priority Date:

Title of invention: **PROCESS OF BLOCKING PLASMODIUM GAMETOCYTOGENESIS AND TRANSMISSION USING JULIPROSOPINE FROM PROSOPIS JULIFLORA.**

The applicant is hereby notified that formality examination of the above-identified application has been carried out in accordance with section 41(7) of the Industrial Property Act, 2001 and according to the report, no defects were identified.

You are hereby invited to note the following requirements:

1. The application shall be published as provided under Section 42 of the Industrial Property Act 2001, either:
   ☐ **after** expiration of 18 months from the filing date or the priority date indicated above, or

   ☐ **before** the expiry of eighteen months at the request of the applicant.

   In either case, you are required to pay publication fee of **Ksh 3000** for a local applicant or **US$150** for a foreign applicant. The publication fee should be accompanied by a copy of this letter with the preferred publication period selected in one of the above check boxes.

2. You are required to file a request for substantive examination as provided under Section 44 (2) of the Act by submitting form IP 8 and paying a fee of **Ksh.5000** for a local applicant or **US$250** for a foreign applicant **within five (5) years from the filing date of this application**. If you do not submit a request for examination as indicated, this application shall be deemed abandoned.

3. At any time before the grant or refusal of a patent you may upon payment of a fee of **Ksh 500** for a local applicant or **US$50** for a foreign applicant convert this patent application to an application for a Utility Model certificate.

4. In order to maintain this application, an annual fee of **Ksh 2000** for a local applicant or **US$300** for a foreign applicant is payable (by submitting Form IP 16) in advance or on the eve of each anniversary of the filing date. A grace period of 6 months is allowed for late payment of the annual fee subject to payment of a surcharge of **Ksh. 3000** or **US$150**. If the fees are not paid as herewith indicated, the application shall be deemed to have been withdrawn or abandoned.

Belinda M. Ilagosa
FOR MANAGING DIRECTOR

NA06 Notification of formality compliance.doc

Page 1 of 2

Pesticide Biochemistry and Physiology 178 (2021) 104912

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Neurotoxic *Zanthoxylum chalybeum* root constituents invoke mosquito larval growth retardation through ecdysteroidogenic CYP450s transcriptional perturbations

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[d] Department of Biochemistry & Molecular Biology, Egerton University, Egerton, Kenya
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Natural products
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Ecdysteroidogenic CYP450s

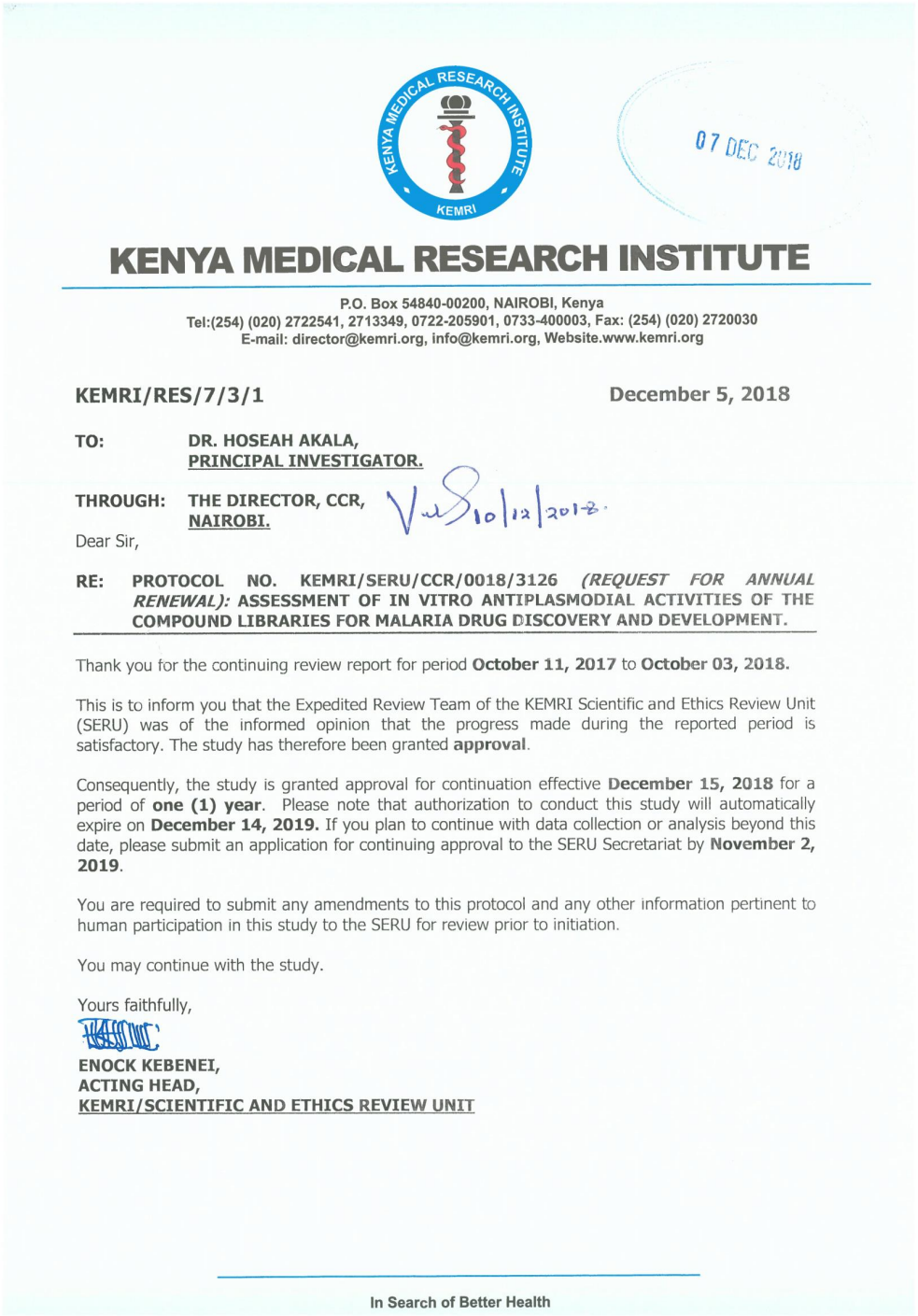
ABSTRACT

Intracellular effects exerted by phytochemicals eliciting insect growth-retarding responses during vector control intervention remain largely underexplored. We studied the effects of *Zanthoxylum chalybeum* Engl. (Rutaceae) (ZCE) root derivatives against malaria (*Anopheles gambiae*) and arbovirus vector (*Aedes aegypti*) larvae to decipher possible molecular targets. We report dose-dependent biphasic effects on larval response, with transient exposure to ZCE and its bioactive fraction (ZCFr.5) inhibiting acetylcholinesterase (AChE) activity, inducing larval lethality and growth retardation at sublethal doses. Half-maximal lethal concentrations (LC_{50}) for ZCE against *An. gambiae* and *Ae. aegypti* larvae after 24-h exposure were 9.00 ppm and 12.26 ppm, respectively. The active fraction ZCFr.5 exerted LC_{50} of 1.58 ppm and 3.21 ppm for *An. gambiae* and *Ae. aegypti* larvae, respectively. Inhibition of AChE was potentially linked to larval toxicity afforded by 2-tridecanone, palmitic acid (hexadecanoic acid), linoleic acid ((*Z,Z*)-9,12-octadecadienoic acid), sesamin, β-caryophyllene among other compounds identified in the bioactive fraction. In addition, the phenotypic larval retardation induced by ZCE root constituents was exerted through transcriptional modulation of ecdysteroidogenic CYP450 genes. Collectively, these findings provide an explorative avenue for developing potential mosquito control agents from *Z. chalybeum* root constituents.

1. Introduction

Renewed interests in search of environmentally friendly alternative insecticides have lately led to the gradual substitution of chemical-based insecticides in global markets. However, as much as the public demand for biocides over their synthetic counterparts continues to increase and Conversely, detailed molecular toxicity studies to demystify the mechanisms of action of various mosquitocidal agents are few. In our recent study, tea proanthocyanidins interfered with mosquito larval growth and reproduction fitness by physiologically disrupting the juvenile hormone biosynthetic pathway (Muema et al., 2017b). Using a yeast two-hybrid reporter system, Lee et al. showed that juvenile hormone

Appendix IX: KEMRI SERU and WRAIR Ethical Review approvals



07 DEC 2018

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 December 5, 2018

TO: **DR. HOSEAH AKALA,**
PRINCIPAL INVESTIGATOR.

THROUGH: **THE DIRECTOR, CCR,**
NAIROBI.

10/12/2018

Dear Sir,

RE: **PROTOCOL NO. KEMRI/SERU/CCR/0018/3126** ***(REQUEST FOR ANNUAL RENEWAL):*** **ASSESSMENT OF IN VITRO ANTIPLASMODIAL ACTIVITIES OF THE COMPOUND LIBRARIES FOR MALARIA DRUG DISCOVERY AND DEVELOPMENT.**

Thank you for the continuing review report for period **October 11, 2017** to **October 03, 2018.**

This is to inform you that the Expedited Review Team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval**.

Consequently, the study is granted approval for continuation effective **December 15, 2018** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **December 14, 2019.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the SERU Secretariat by **November 2, 2019**.

You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to the SERU for review prior to initiation.

You may continue with the study.

Yours faithfully,

ENOCK KEBENEI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

13 MAR 2019

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 March 11, 2019

TO: **DR. JAMES MUTUNGA**
PRINCIPAL INVESTIGATOR

for **THROUGH:** **THE DIRECTOR, CCR** forwarded 13/3/2019.
NAIROBI

Dear Sir,

RE: **KEMRI/SERU/CCR/0111/3777 *(RESUBMISSION OF INITIAL SUBMISSION)*: ANTI-MALARIAL DRUG DELIVERY TO INFECTED LABORATORY REARED *ANOPHELES GAMBIAE* USING SUGAR BAITS TO TARGET PLASMODIUM MOSQUITOES**

Reference is made to your letter dated February 27, 2019. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on March 07, 2019.
The committee noted the following attached documents:

a. Comments letter dated 06 Dec 2018
b. Revised protocol versions 003 dated 15 Jan 2019 tracked and clean versions.
c. Copies of current approvals for WRAIR#1919 SSC1330
d. Copy of the Protocol for WRAIR #1919 SSC 1330
e. A copy of the Informed consent form for WRAIR #1919 SSC 1330
f. A letter from the PI of WRAIR#1919 SSC 1330 authorizing use of samples.

This is to inform you that the Committee noted that the issues raised at the 281st Committee C meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **November 29, 2018**, have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **March 11, 2019** for a period of one year. Please note that authorization to conduct this study will automatically expire on **March 10, 2020.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **January 28, 2020**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued. You may embark on the study.

Yours faithfully,

ENOCK KEBENEI
THE ACTING HEAD
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
503 ROBERT GRANT AVENUE
SILVER SPRING, MD 20910-7500

REPLY TO ATTENTION OF

MCMR-UWZ-C 1 April 2019

MEMORANDUM FOR James Mutunga, PhD, Entomology and Vector Biology Branch, U.S. Army Medical Research Directorate, Kenya, P.O. Box 54-40100, Kisumu, Kenya

SUBJECT: Project Qualifies as Research Not Involving Human Subjects, **WRAIR #2606**

1. A determination was made that the project **WRAIR #2606**, titled, "Anti-Malarial Drug Delivery to Infected Laboratory Reared *Anopheles gambiae* using Sugar Baits to Target *Plasmodium* in Mosquitoes," (Version 004, dated 28 February 2019) does not require review by the WRAIR Institutional Review Board (IRB) in accordance with WRAIR Policy Letter #12-09, as the project involves the use of anonymized human blood samples from WRAIR# 1919 for culturing parasites, where the Principal Investigator (PI) will not have access to the links to identifiable information. As the PI will not have access to identifiable information; this research activity does not meet the definition of research involving human subjects and 32 CFR 219 does not apply.

2. The primary objective of this study is to assess anti-malarial drug delivery to *Anopheles gambiae* using sugar baits targeting *Plasmodium* transmission in mosquitoes. Anonymized blood from WRAIR# 1919 will be used to culture parasites as part of activity #2. Otherwise, all other activities under this protocol will be conducted using mosquitoes.

Activity #2 cannot be conducted until amendment #3 for WRAIR# 1919 has been submitted, reviewed and approved to add the work under this protocol.

3. This study is sponsored by WRAIR. Funding is provided by the Office of the Secretary of Defense (OSD). This effort is being funded by the OSD which was awarded via WRAIR, through the MIDRP program.

4. The USAMRD-K Director determined this project to be scientifically feasible and valid; militarily relevant; and appropriately resourced on 12 March 2019.

5. The Kenya Medical Research Institute, Scientific Ethics Review Unit approved this protocol on 11 March 2019.

6. The PI has the responsibility to obtain all business agreements prior to initiation of any work with partners/collaborators or contracted services. This includes any transfer of data or specimens. Failure to obtain business agreements prior to initiation could result in sanctions or disciplinary actions for the USAMRD-K Director and the PI. The IRB and the Human Subjects Protection Branch (HSPB) will review business agreements as part of monitoring visits to ensure they were obtained as required and report to the WRAIR Commander as to adherence to this requirement. Please seek clarification from the Office of Research Technology and Applications (ORTA).

7. No additional information is required at this time. However, should the PI gain access to the information linking the data with subjects from WRAIR# 1919, the submitted project will need an

independent determination by the Chair, WRAIR IRB, or the Director, Human Subjects Protection Branch (HSPB), as to whether or not the investigator is engaged in human subjects research, and whether or not the WRAIR IRB review and approval are required. The HSPB reserves the right to review the project records to re-assess the determination of research not involving human subjects. The WRAIR HSPB also reserves the right to review the project records and re-assess the research not involving human subjects determination as part of post approval compliance monitoring. The PI is responsible for maintaining records that confirm that the executed activities match the project that was evaluated and found to be research not involving human subjects.

8. The point of contact for this action is the undersigned, at 301-319-9438 or teresa.r.soderberg.civ@mail.mil.

TERESA R. SODERBERG, M.A., RAC
Exempt Determination Official
Human Subjects Protection Branch
Walter Reed Army Institute of Research

CF:
Patrick McCardle, MAJ, MS
Nathaniel Copeland, MAJ, MC
John Distelhorst, MAJ, MC
Douglas Shaffer, MD
Stacey Gondi
ORTA, WRAIR

DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
503 ROBERT GRANT AVENUE
SILVER SPRING, MD 20910-7500

REPLY TO
ATTENTION OF

FCMR-UWZ-C

MEMORANDUM FOR Ben Andagalu, M.S., MSc, Kenya Medical Research Institute (KEMRI)/Walter Reed Project (WRP), P.O. Box 54, 40100, Kisumu, Kenya

SUBJECT: Commander Approval Authorization of Amendment #3 to the Minimal Risk Human Subjects Research Protocol, **WRAIR #1919**

1. Amendment #3 (Protocol Version 2.8, dated 1 April 2019) for the protocol **WRAIR #1919**, entitled, "Human Blood Collection for the *In vitro* Culture of Malaria Parasites," and supporting information have been submitted in accordance with applicable Walter Reed Army Institute of Research (WRAIR) and Federal policies, procedures, and guidance.

2. The WRAIR Institutional Review Board (IRB) Chair Designee approved amendment #3 (Protocol Version 2.8, dated 1 April 2019) on 9 May 2019. (See Enclosure)

3. The following human subjects protection related document pending at the time of the WRAIR IRB approval, has been received by the Human Subjects Protection Branch (HSPB): KEMRI, Scientific and Ethical Review Unit (SERU), approval, dated 2 July 2019, (Received: 11 July 2019).

4. As a reminder, the WRAIR expiration date is **23 May 2020**. The Principal Investigator is responsible for submitting a continuing review report to the WRAIR HSPB in time for the report to be reviewed and approved by the KEMRI SERU, and reviewed and accepted by the WRAIR IRB prior to the respective study expiration dates in order for work to continue without interruption. A closeout report or request for extension must be submitted to the WRAIR HSPB not later than **23 May 2022**. No changes, amendments, or addenda may be made to the protocol without prior review and approval by the KEMRI SERU and the WRAIR IRB. As part of the WRAIR IRB's responsibility to confirm research is being conducted in a manner where the conditions of the federal regulations, Department of Defense Instruction, IRB and institutional policies are being met, this protocol may be selected for Post-Approval Compliance Monitoring.

5. As there are no outstanding human subjects protection issues, approval authorization is granted for amendment #3 (Protocol Version 2.8, dated 1 April 2019) to this minimal risk protocol.

FCMR-UWZ-C
SUBJECT: Commander Approval Authorization of Amendment #3 to the Minimal Risk Human Subjects Research Protocol, **WRAIR #1919**

6. The point of contact for this action is Teresa R. Soderberg, M.A., RAC, at (301) 319-9438 or at teresa.r.soderberg.civ@mail.mil.

TEYHEN.DEYDRE.SMYTH.1076991975
Digitally signed by TEYHEN.DEYDRE.SMYTH.1076991975
Date: 2019.07.13 13:45:13 -04'00'

Encl
WRAIR IRB Approval,
9 May 2019

DEYDRE S. TEYHEN
COL, SP
Commanding

CF:
WRAIR Deputy Commander
John Distelhorst, LTC, MC
Nathaniel Copeland, MAJ, MC
Ben Andagalu, M.D., MSc
Kisumu Regulatory Affairs
Jody Ference, M.S., CCRA, CIP, CIM
Tibor Tuzson, MD, MS

Appendix X: JKUAT research approval

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
NAIROBI – 00200
KENYA

TEL: 254-067-580001, 580003,580005 Ext. 1655
Email: director@bps.jkuat.ac.ke

REF BPS/ HSB411-2550/2017 **6TH SEPTEMBER, 2018**

JACKSON MBITHI MUEMA
C/o SOBMS
JKUAT

Dear Mr. Mbithi,

RE: APPROVAL OF RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that the Board of Postgraduate Studies has approved your PhD. research proposal entitled: **"DISCOVERY AND MECHANISTIC CHARACTERIZATION OF NOVEL PLANT- DERIVED MALARIA TRANSMISSION- BLOCKING AGENTS"**. The following are your approved supervisors:-

4. Dr. Joel Bargul
5. Dr. Meshack Obonyo
6. Dr. Ramadhan Mwakubambanya
7. Dr. James Mutunga

Yours Sincerely

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Copy to: Dean SOBMS