The Effectiveness of Varying Combination Ratios of A. cordifolia and M. indica against Field and Laboratory Strains of P. falciparum In Vitro

Yakubu Jibira,1 Elizabeth Cudjoe,2 Frederick M. Tei-Maya,2 Benjamin Ayensu,3 and Linda E. Amoah1

1Department of Pharmacology, College of Health Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana 2Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Accra, Ghana 3Immunology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

Correspondence should be addressed to Linda E. Amoah; lamoah@noguchi.ug.edu.gh

Received 25 July 2020; Revised 3 October 2020; Accepted 28 October 2020; Published 16 November 2020

Background. Drug resistance in malaria is a global problem, with reports of Plasmodium parasites resistant to the current first-line antimalarial drug, artemisinin, expanding from Southeast Asia to Africa. There is therefore an urgent need to identify new drug candidates that will be effective against the existing malaria parasites. Drug combination therapy presents a myriad of advantages over monotherapy including delayed onset of resistance, potentiation, and synergism. This present study explored the effectiveness of combinations of aqueous extracts of Alchornea cordifolia (A. cordifolia) and Mangifera indica (M. indica) at clearing both laboratory and field isolates of P. falciparum. Methods. Synchronized ring stage cultures of field (FA08) and laboratory strains (NF54 and CamWT_C580Y) of P. falciparum were subjected to combinations of different concentrations and ratios of aqueous extracts of A. cordifolia and M. indica. The growth inhibition of the individual plant extracts and their combinatorial effects were studied in vitro using SYBR Green I drug assay. Results. The A. cordifolia extract exhibited 50% inhibitory concentration (IC 50) of 2.71, 7.80, and 3.56 μg/mL against the NF54, CamWT_C580Y, and FA08 parasite strains, respectively. Mangifera indica exhibited IC 50 of 18.11, 20.08, and 10.23 μg/mL against the NF54, CamWT_C580Y, and FA08 parasite strains, respectively. Additive, synergistic and antagonistic interactions were observed at different combinations of A. cordifolia and M. indica extracts. Conclusion. A combination product containing A. cordifolia and M. indica has the potential to serve as an effective antimalarial as majority of the tested combinations of aqueous extracts of A. cordifolia and M. indica extracts exhibited synergistic effects in vitro against the NF54, CamWT_C580Y, and FA08 P. falciparum strains.
natural organic origin and minimal known side effects [7]. Traditional herbal remedies have been adopted in treating malaria patients since ancient times, with over 1200 plant species from 160 families used for the traditional treatment of malaria [6, 8].

*Mangifera indica* (*M. indica*) is a common plant component of local herbal medicines on the Ghanaiian market [9]. *Mangifera* spp. is a large genus of evergreen trees, distributed in tropical and subtropical parts of Southeast Asia. The popularity of the plant stems from its many medicinal attributes such as antiviral, antibacterial, and antiplasmodial [7, 10, 11]. Although there is a report of the antimalarial properties of mangiferin, a major component of *M. indica* [12], the active antimalarial components of *M. indica* have not been characterized and activity-guided bioassays are needed to identify the active compounds [13].

*Alchornea cordifolia* (*A. cordifolia*) is a West African plant that belongs to the family of Euphorbiaceae [14]. The active compound in the leaves of *A. cordifolia* has been found to be elagic acid, which is able to inhibit the growth of *P. falciparum* without any cytotoxicity [15].

Herbal antimalarial products, which are the most popular herbal products on the Ghanaiian market, are usually composed of a mixture of a variety of herbal extracts [16]. Although the independent aqueous extracts of *A. cordifolia* and *M. indica* exhibit very good antimalarial activity against *P. falciparum* parasites [11, 17–19], knowledge on the antimalarial activity of different combinations of these two extracts is lacking. In this study, we identify the effects of different combinations of *A. cordifolia* and *M. indica* on laboratory and field isolates of *P. falciparum*.

2. Methods

2.1. Identification and Preparation of Herbal Extracts. The herbal extracts used in this study have previously been described [12]. Briefly, fresh leaves of *A. cordifolia* were obtained from Apooh in the Shama Ahanta East district of the Western Region (4° 54' 26.6405'' N, 1° 49' 6.6002'' W) and *M. indica*, from Adenta (5° 42' 32.8931'' N, 0° 10' 13.2384'' W) in the Adentan Municipality of the Greater Accra region of Ghana. The leaves were identified at the University of Ghana herbarium, Accra, and the herbarium at the Centre for Plant Medicine Research (CPMR), Mampong. The leaves were then air dried and ground into powder using a blender. Each set of ground leaves (21.5 g of *A. cordifolia* and 32 g of *M. indica*) were boiled in 450 mL of distilled water at 100° C for an hour. The solutions were left to cool at room temperature for 18 hours and then filtered using Whatman™ 54 filter paper. The filtered solutions were finally lyophilized using a Labconco™ Freeze Dryer. Stock concentrations of 50 mg/mL were prepared for both extracts by dissolving 500 mg of the lyophilized extract in 10 mL distilled water. The stock solutions were subsequently filtered through a 0.2 μm Acodisc™ syringe filter and used immediately or stored at -20° C.

2.2. Culturing of Plasmodium Parasites. Asexual cultures of NF54 (MRA-1250: chloroquine sensitive), CamWT_C580Y (MRA-1250: artemisinin sensitive), and FA08 (Ghanaian culture adapted field isolate) were maintained in vitro using a modified method of Mustofa [17] and similar to Amoah et al. [20]. Briefly, the parasites were individually cultured at 4% hematocrit (O+ red blood cells (RBCs)) in complete parasite media (CPM: RPMI 1640 supplemented with 25 mM HEPES, 2 mL-glutamine, 24.1 mM NaHCO₃, 11.1 mM glucose, 50 μg/mL gentamycin, and 0.5% Albumax II) in a T75 culture flask. The cultures were maintained in an incubator set at 37° C with daily media change with CPM and exchange of gas (92.5% nitrogen, 5.5% carbon dioxide, and 2% oxygen).

Synchronized ring stage parasites were obtained by treating a culture containing more than 5% ring stage parasites with a solution of 5% sorbitol for 10 minutes at room temperature. Two days (48 hours) after synchronization, the cultures, which were predominantly ring stage parasites were plated at 2% for the SYBR Green 1 assay.

2.3. SYBR Green I Asexual Parasite Drug Assay. A protocol similar to that described by Cudjoe et al. [11] and Smilkstein et al. [21] with some revisions was used to determine the inhibitory effects of the aqueous extracts of *A. cordifolia* and *M. indica* on different *P. falciparum* parasites. A schematic of the plate was set up in Additional file 1. Briefly, 25 μL of a fixed concentration (40, 20, 10, 5, and 0 μg/mL) of *A. cordifolia* and another 25 μL of a fixed concentration (100, 40, 20, 10, and 0 μg/mL) of *M. indica* were dispensed in triplicate into the wells of a 96-well tissue culture plate (Additional file S1). The aqueous extracts of *A. cordifolia* and *M. indica* used in this study have been previously described [11]. Positive control wells were filled with 50 μL of different concentrations (400–1 ng/mL) of the reference drug artesunate (AS) (Additional file S1). Two plates containing different mixtures of *A. cordifolia* and *M. indica* extracts and AS were set up for each parasite strain. A series of untreated infected RBCs set at 1%-0.25% parasitaemia was also set up in triplicates to serve as negative controls for the assay (Additional file S1). Each of the remaining wells were then supplemented with 50 μL of ring stage-infected RBC (IRBCs) set at 1% parasitemia (either NF54, FA8, or CamWT_C580Y) and 4% hematocrit in CPM. The plates were then placed into a modular incubating chamber, gassed for 6 minutes and then incubated for 72 hours at 37° C. Two technical replicate plates were set up for each assay.

The plates were subsequently wrapped in aluminum foil and frozen overnight at -20° C. The plates were thawed at room temperature, and each well was then filled with 100 μL of buffered SYBR Green (2x SYBR Green 1 dye in 20 mM Tris-HCl, pH 7.5 supplemented with 5 mM EDTA, 0.08% Triton X-100, and 0.008% saponin in PBS). The plate was wrapped again in aluminum foil, incubated at 37° C for 1 hour, and fluorescence was then read on a microplate reader at 497 nm excitation and 530 nm emission.

2.4. Statistical Analysis. For the SYBR Green 1 drug assays, the data obtained from the herbal extract-treated uninfected RBC was used as the background and subtracted from the corresponding infected RBC data set.
Data was converted into % inhibition using the formula:

\[
\%\text{Inhibition} = 100 \times \left[ 1 - \left( \frac{X - \text{min}}{\text{Max} - \text{min}} \right) \right],
\]

where \(X\) is the signal at a given concentration of the inhibitor, \(\text{min}\) is the signal with 100% inhibition, and \(\text{Max}\) is the signal with no inhibition.

2.5. Isobologram analysis. The growth inhibition caused by \(A.\ cordifolia\), and \(M.\ indica\) extracts and AS were individually normalized as percentages and plotted against the log concentration of the drugs. The data obtained was analysed using Compusyn (Compusyn Software, Combosyn, Inc., PD Science LLC, USA). The software is based on the Chou-Talalay method for drug combination (based on median-effect equation) which provides combination index- (CI-) isobologram equation that gives quantitative determination of drug interaction, where \(\text{CI} < 1\), \(\text{CI} > 1\), and \(\text{CI} = 1\) denotes synergism, antagonism, and additive effect, respectively [22]. The resulting sigmoidal dose response curves used to calculate the 50% inhibitory concentration (IC\(_{50}\)) [22, 23]. Dose-response curves were also obtained and analyzed after the coadministration of \(A.\ cordifolia\) and \(M.\ indica\) extracts in fixed combination ratios. For each combination, the IC\(_{50}\) (experimental), CI, and its associated fraction affected (Fa) were evaluated by a quantitative diagnostic plot (Fa-CI) analysis of the log dose-response curve obtained using the three formulas below:

\[
\frac{\text{Fa}}{\text{Fu}} = \left[ \frac{D}{\text{Dm}} \right]^m,
\]

\[
\log \left[ \frac{\text{Fa}}{\text{Fu}} \right] = m \log D - m \log \text{Dm},
\]

\[
\text{CI} = \sum_{j=1}^{n} \left[ \frac{D}{\text{Dx}} \right]^j,
\]

where \(\text{Fa}\) is the fraction affected, \(\text{Fu}\) is the fraction unaffected, \(D\) is the dose required to produce Fa, \(\text{Dm}\) is the the median dose effect (IC\(_{50}\)); \(m\) is the dynamic order (sigmoidicity), and \(\text{Dx}\) is the dose of each drug alone that exerts X % inhibition.

\(P\) values for statistical significance were set at 0.05.

3. Results

The aqueous extract of \(A.\ cordifolia\) primarily displayed a sigmoid-shaped dose-response relationship (Figure 1) with an approximate IC\(_{50}\) against NF54, CamWT\(_{C580Y}\), and FA08 \(P.\ falci-parum\) parasite strain of 2.71, 7.80, and 3.56 \(\mu\)g/mL (Table 1). The IC\(_{50}\) values for \(M.\ indica\) were 18.11, 20.08, and 10.23 \(\mu\)g/mL against the NF54, CamWT\(_{C580Y}\), and FA08 \(P.\ falci-parum\) parasite strains, respectively, were lower than those obtained for \(A.\ cordifolia\).

The activity of \(A.\ cordifolia\) was relatively similar amongst the three parasite strains but was highest against the NF54 strain, whilst the activity of \(M.\ indica\) was highest in the FA08 strain (Table 1, Additional file Figure S2).

Taking cognizance of the drug-interaction assay, the combinations of \(A.\ cordifolia\) with \(M.\ indica\) were synergistic, antagonistic, and additive at different combinations ratios (Table 1). The activity of combinations of \(A.\ cordifolia\) with \(M.\ indica\) against the CamWT\(_{C580Y}\) and NF54 strains showed CI of less than 1 suggesting synergy except for ratios of when the combinations were at a 10:1 and 20:1 ratio (NF54) or 10:1 and 20:1 against the CamWT\(_{C580Y}\) parasites. The degree of synergism was stronger at a 1:4 ratio of \(A.\ cordifolia:\) with \(M.\ indica\) (CI = 0.167) followed by the 1:2 ratio (CI = 0.192) and finally the 2:1 (CI = 0.300) against CamWT\(_{C580Y}\) parasites (Table 1).

A significant suppression of parasite growth was observed when the NF54 parasite strain was treated with 19.67 \(\mu\)g/mL of \(A.\ cordifolia\) and 98.98 \(\mu\)g/mL for \(M.\ indica\). Similarly, a significant suppression in the growth of the FA8 strain was observed when treated with 27.62 \(\mu\)g/mL of \(A.\ cordifolia\) and 13.73 \(\mu\)g/mL \(M.\ indica\). A significant reduction in the growth of the CamWT\(_{580Y}\) parasite strain was observed when the parasites were treated with 150.65 \(\mu\)g/mL of \(A.\ cordifolia\) and 169.48 \(\mu\)g/mL \(M.\ indica\) (Table 2). In addition, combinations of \(A.\ cordifolia\) with \(M.\ indica\) at 2:1, 1:1, and 1:2 ratios showed synergistic effects against the NF54 and CamWT\(_{580Y}\) parasites and antagonistic effects at ratios of 2:1, 1:1, 1:2, and 10:1 against NF54, FA8, and CamWT\(_{580Y}\). The combination caused more significant \(P < 0.05\) suppression in parasitaemia burden with estimated dose of 20.07 \(\mu\)g/mL against NF54, 44.28 \(\mu\)g/mL against FA8, and 31.26 \(\mu\)g/mL against CamWT\(_{580Y}\) at 1:2, 1:1, and 2:1 ratios, respectively.

4. Discussion

Sustainable malaria control requires a combination of interventions and tools as well as research and development of enhanced strategies including vaccines, drugs, diagnostics, and vector management approaches. Antimalarial drugs such as CQ, SP, quinine, and recently the artemisinins have played a vital role in malaria control globally [24]. These drugs mainly target the erythrocytic phase of the infection, which is the phase of infection that presents signs and symptoms [25].

The rapid development of resistance to these commonly used antimalarial drugs by the malaria parasite has led to the recommendation that drug therapy should target multiple targets, so as to reduce the development of resistance. Suggesting the need for the use of drug combinations or a single drug that has multiple targets. There is also a major challenge existing in the supply and use of antimalarial drug combination therapies, particularly in Africa. The antimalarial activities of aqueous extracts of \(A.\ cordifolia\) and \(M.\ indica\) have recently been confirmed against a number of \(P.\ falci-parum\) isolates [11]. This present study goes further to investigate the antimalarial activity of combinations of aqueous extracts of \(A.\ cordifolia\) and \(M.\ indica\) in vitro.

The aqueous extracts of both \(A.\ cordifolia\) and \(M.\ indica\) clearly suppressed the growth of all the three isolates of \(P.\ falci-parum\) used in the study. This supports previous reports of extracts of both \(A.\ cordifolia\) and \(M.\ indica\) exhibiting
antimalarial activity and also supports the traditional use of various parts of *A. cordifolia* and *M. indica* in the treatment of malaria [18, 26, 27]. The IC\textsubscript{50} values reported for NF54 and CamWT\_C580Y are similar to a recent report that used a slightly different plate set up for the sybrgreen 1 assay [11]. There was only one tested ratio of *A. cordifolia* and *M. indica* that exhibited synergistic effects against all the three parasite strains and also one tested ratio that yielded antagonistic effects against all the three parasite strains tested. This demonstrates that extensive studies using multiple parasite strains are required to identify the most appropriate combination ratio for *A. cordifolia* and *M. indica* as it is possible that a combination ratio that exerts high levels of synergistic effects on a tested parasite strain can be antagonistic against a different parasite strain. The observation that different combination ratios can result in different effects support previous reports where different amounts of cepharanthine combined with atovaquone and lumefantrine resulted in synergistic and additive effects against the W2 strain of *P. falciparum* [25].

The mode of action of *A. cordifolia* against *P. falciparum* is likely different from that of *M. indica* as the activity of *A. cordifolia* was highest against the NF54 strain but the activity of *M. indica* was highest in the FA08 strain (Table 1). The possibility of these two extracts having different modes of
action against the malaria parasite make them ideal combination partners for the treatment of malaria. The in vitro anti-malarial interactions of *A. cordifolia* in combination with *M. indica* had less than 1 combination index (CI) against CamWT_580Y depicting a strong synergy. Also, values obtained with five *A. cordifolia* combinations indicate a synergistic interaction of the *M. indica* against the NF54 strain. Artemisinin-based combination therapy (ACT) is now the mainstay in the treatment of malaria in Africa due its efficacy in the rapid clearance of symptoms and parasites and profound efficacy and low probability of drug-resistance development [24, 28].

This present study focused on the combination of aqueous extracts of *A. cordifolia* and *M. indica*, with the aim of developing novel combinational therapies to preempt the advancement of resistance to existing antimalarial drugs. As with ACT treatment, crude herbal extracts and their mixtures are expected to delay the onset of antimalarial drug resistance relative to single-component therapies due to the likelihood of the variant constituent components acting on different drug targets within the parasite [29]. Although the actual mechanism of action of either *A. cordifolia* or *M. indica* as antimalarial is unknown, the fact that growth inhibition of different parasite strains was effective suggests that appropriate combinations of *A. cordifolia* and *M. indica* have the potential to be used as potent schizonticides against a variety of *P. falciparum* parasites.

### 5. Conclusions

A combination product containing *A. cordifolia* and *M. indica* has the potential to serve as an effective antimalarial as majority of the tested combinations of aqueous extracts of *A. cordifolia* and *M. indica* extracts exhibited synergistic effects in vitro against the NF54, CamWT_C580Y, and FA08 *P. falciparum* strains.

### Data Availability

The data used to support the findings of this study are included within the article.

---

**Table 1**: The drug combination of *A. cordifolia* with *M. indica* against NF54, FA08, and CamWT_C580Y *Plasmodium* parasite strains.

| Drugs   | HE ratio | IC₅₀ (μg/mL) | CI (Fa = 95) | Interaction | Correlation coefficient (r) |
|---------|----------|--------------|--------------|-------------|----------------------------|
| NF54    |          |              |              |             |                            |
| Ac      | N/A      | 2.71         | N/A          | N/A         | 0.988                      |
| Mi      | N/A      | 18.11        | N/A          | N/A         | 0.988                      |
| Ac + Mi | 02:01    | 4.98         | 0.75         | Syn         | 0.999                      |
| Ac + Mi | 01:02    | 8.58         | 0.58         | Syn         | 1                          |
| Ac + Mi | 01:01    | 6.43         | 0.78         | Syn         | 1                          |
| Ac + Mi | 04:01    | 4.52         | 0.72         | Syn         | 1                          |
| Ac + Mi | 01:04    | 10.04        | 0.66         | Syn         | 1                          |
| Ac + Mi | 01:05    | 10.54        | 0.61         | Syn         | 1                          |
| Ac + Mi | 10:01    | 13.60        | 2.56         | Anta        | 0.995                      |
| Ac + Mi | 20:01    | 16.40        | 2.83         | Anta        | 1                          |
| FA08    |          |              |              |             |                            |
| Ac      | N/A      | 3.55         | N/A          | N/A         | 0.888                      |
| Mi      | N/A      | 10.23        | N/A          | N/A         | 0.934                      |
| Ac + Mi | 02:01    | 770.26       | 3.348        | Anta        | 0.134                      |
| Ac + Mi | 01:02    | 10.92        | 3.03         | Anta        | 0.786                      |
| Ac + Mi | 01:01    | 11.99        | 2.41         | Anta        | 0.89                       |
| Ac + Mi | 04:01    | 20.73        | 15.34        | Anta        | 1                          |
| Ac + Mi | 01:04    | 10.74        | 0.90         | Syn         | 1                          |
| Ac + Mi | 10:01    | 19.81        | 2.65         | Anta        | 1                          |
| Ac + Mi | 20:01    | 22.33        | 2.29         | Anta        | 1                          |
| CamWT   |          |              |              |             |                            |
| Ac      | N/A      | 7.80         | N/A          | N/A         | 0.933                      |
| Mi      | N/A      | 20.08        | N/A          | N/A         | 0.938                      |
| Ac + Mi | 02:01    | 13.47        | 0.30         | Syn         | 1                          |
| Ac + Mi | 01:02    | 11.38        | 0.19         | Syn         | 1                          |
| Ac + Mi | 01:01    | 12.93        | 0.32         | Syn         | 1                          |
| Ac + Mi | 04:01    | 17.42        | 0.79         | Syn         | 1                          |
| Ac + Mi | 01:04    | 10.18        | 0.17         | Syn         | 1                          |
| Ac + Mi | 01:05    | 13.72        | 0.55         | Syn         | 1                          |
| Ac + Mi | 10:01    | 14.57        | 1.05         | Add         | 0.976                      |
| Ac + Mi | 20:01    | 4.91         | 6.75         | Anta        | 1                          |

IC₅₀ (μg/mL) signifies potency, and r represents linear correlation coefficient. CI = 1, <1, and >1 depict additive effect, synergism, and antagonism, respectively. Drug combinations were generated from the Compusyn software report. Ac: *A. cordifolia* extract; Mi: *M. indica* extract; CamWT: CamWT_580Y; Syn: synergy; Add: additive; Anta: antagonistic.

**Table 2**: The Compusyn drug combination estimated dose of *A. cordifolia* with *M. indica* on the three parasite strains.

| HE/combo | CI value | Dose Ac | Dose Mi |
|----------|----------|---------|---------|
| Ac       | 19.67    | 98.98   |
| Mi       | 13.73    | 27.62   |
| FA8      | 20.84    | 25.44   | 14.54   |
| NF54     | 5.59     | 9.84    | 6.69    |
| A1 : M1  | 12.81    | 12.81   |
| A10 : M1 | 49.41    | 4.94    |

Cl = 1, <1, and >1 depict additive effect, synergism, and antagonism, respectively. Ac: *A. cordifolia*; Mi: *M. indica* extract; CamWT: CamWT_580Y; syn: synergy; add: additive; anta: antagonistic.
Conﬂicts of Interest

The authors declare that they have no conﬂicts of interest.

Supplementary Materials

Additional file Figure S1: drug assay plate set up. Additional file Figure S2: graphical representation of combination growth inhibition data. (Supplementary Materials)

References

[1] WHO, World malaria report Geneva, World Health Organisation, 2019.

[2] NMCP, The National Malaria Control Programme performance review 2011, NMCP, 2013.

[3] P. Ambroise-Thomas, “The tragedy caused by fake antimalarial drugs,” Mediterr J Hematol Infect Dis., vol. 4, no. 1, article e2012027, 2012.

[4] A. K. Rai, N. Bhaskar, and V. Baskaran, “Effect of feeding lipids recovered from ﬁsh processing waste by lactic acid fermentation and enzymatic hydrolysis on antioxidant and membrane bound enzymes in rats,” Journal of Food Science and Technology, vol. 52, no. 6, pp. 3701–3710, 2015.

[5] M. L. Willcox and G. Bodeker, “Traditional herbal medicines for malaria,” BMJ, vol. 329, no. 7475, pp. 1156–1159, 2004.

[6] M. Parvez, “Pharmacological activities of mango (Mangifera indica): a review,” Journal of Pharmacognosy and Phytochemistry, vol. 5, no. 1, 2016.

[7] T. Ngarivhume, C. I. E. A. van’t Klooster, J. T. de Jong, and J. H. van der Westhuizen, “Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe,” Journal of Ethnopharmacology, vol. 159, pp. 224–237, 2015.

[8] P. Saotoing, T. Vroumsia, Tchobsala, F. F. Tchuenguem, N. A. Taudon, and J. Messi, “Lymphoid agents and chemotherapy. In vitro and in vivo combination of cepharanthine with anti-malarial drugs,” Malaria Journal, vol. 16, no. 1, p. 103, 2017.

[9] A. Valentin, Mustofa, F. Benoit-Vical, Y. Pelissier, D. Koné-Bamba, and M. Malliè, “Antiplasmodial activity of plant extracts used in west African traditional medicine,” Journal of Ethnopharmacology, vol. 73, no. 1-2, pp. 145–151, 2000.

[10] J.-T. Banzouzi, R. Prado, H. Menan et al., “In vitro antiplasmodial activity of extracts of Alchornea cordifolia and identiﬁcation of an active constituent: ellagic acid,” Journal of Ethnopharmacology, vol. 81, no. 3, pp. 399–401, 2002.

[11] C. F. Eliakim-Ikechukwu and E. B. Riman, “The effect of aqueous ethanolic extract of Alchornea cordifolia leaf on the histology of the aorta of Wistar rats,” Nigerian Journal of Physiological Sciences, vol. 24, no. 2, pp. 149–151, 2009.

[12] E. M. Fevre, G. Barnish, P. Yamokgul, and W. Rooney, “Antiplasmodial activity of ethanolic extracts used in west African traditional medicine, Journal of Parasitology Research, vol. 7, no. 3, Article ID 784095, pp. 1071–1079, 2010.

[13] P. N. Soh, B. Witkowski, D. Olagnier et al., “In vitro and in vivo properties of ellagic acid in malaria treatment,” Antimicrobial agents and chemotherapy, vol. 53, no. 3, pp. 1100–1106, 2009.

[14] M. L. Willcox and G. Bodeker, “Antiplasmodial activity of plant extracts used in west African traditional medicine,” Journal of Ethnopharmacology, vol. 73, no. 1-2, pp. 145–151, 2000.

[15] P. Saotoing, T. Vroumsia, Tchobsala, F. F. Tchuenguem, N. A. Taudon, and J. Messi, “Medicinal plants used in traditional treatments for malaria in Cameroon,” Journal of Ecology and the Natural Environment, vol. 3, no. 3, pp. 104–117, 2011.

[16] L. E. Amoah, C. Kakaney, B. Kwansa-Bentum, and K. A. Kusi, “Activity of herbal medicines on Plasmodium falciparum gametocytes: implications for malaria transmission in Ghana,” PLoS One, vol. 10, no. 11, article e0142587, 2015.

[17] M. Smilkstein, N. Srilalajaroen, J. X. Kelly, P. Wilairat, and M. Riscoe, “Simple and inexpensive ﬂuorescence-based technique for high-throughput antimalarial drug screening,” Antimicrobial Agents and Chemotherapy, vol. 48, no. 5, pp. 1803–1806, 2004.

[18] T. C. Chou, “Drug combination studies and their synergy quantiﬁcation using the Chou-Talalay method,” Cancer Research, vol. 70, no. 2, pp. 440–446, 2010.

[19] H. Matthews, J. Deakin, M. Rajab, M. Idris-Usman, and N. J. Nirmalan, “Investigating antimalarial drug interactions of ethemet dihydrochloride hydrate using CalcuSyn-based interactivity calculations,” PLoS One, vol. 12, no. 3, article e0173303, 2017.

[20] D. Menard and A. Dondorp, “Antimalarial drug resistance: a threat to malaria elimination,” Cold Spring Harb Perspect Med, vol. 7, no. 7, article a025619, 2017.

[21] C. Desgrousas, J. Dormoi, C. Chapus, E. Ollivier, D. Parzy, and N. Taudon, “In vitro and in vivo combination of cephem antibiotics with anti-malarial drugs,” Malaria Journal, vol. 13, no. 1, p. 90, 2014.

[22] A. Nnamdi, E. Ettebong, and K. Davis, “Antiplasmodial and antioxidant activities of methanolic leaf extract and fractions of Alchornea cordifolia,” Journal of HerbMed Pharmacology, vol. 6, pp. 171–177, 2017.

[23] E. E. Ezekoekwe, A. C. Ene, and C. U. Igwe, “In vivo antiplasmodial effect of ethanol and aqueous extracts of Alchornea cordifolia,” Biochem Anal Biochem, vol. 4, 2015.

[24] A. M. Dondorp, F. Nosten, P. Yi et al., “Artesinin resistance in Plasmodium falciparum malaria,” The New England Journal of Medicine, vol. 361, no. 5, pp. 455–467, 2009.

[25] P. Rasoanaivo, C. W. Wright, M. L. Willcox, and B. Gilbert, “Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions,” Malar Journal, vol. 10, Suppl 1, p. S4, 2011.