Sahel Journal of Life Sciences FUDMA (SAJOLS)
March 2025 Vol. 3(1): 384-390
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: *https://doi.org/10.33003/sajols-2025-0301-47*

Research Article

Ex Vivo Protocol for Evaluating Anti-Schizont Activities and Phytochemical Analysis of *Zingiber officinale* Against *Plasmodium falciparum*

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ABSTRACT

The emergence of antimalarial drug resistance has spurred interest in the exploration of natural alternative therapeutic agents. This study aimed to optimise the ex vivo protocol for evaluating the *anti-schizont* activity of *Zingiber officinale* (ginger) extract against *Plasmodium falciparum*. The cold maceration method was used to obtain the extract. The schizont-inhibitory potential of varying concentrations (1.56 to 100 µg/ml) of ginger extract was assessed in a 96-well plate setup. The evaluation of parasitaemia and schizont count was conducted by Giemsa staining and microscopic examination. The results indicated that the ginger extract exhibited dose-dependent inhibition of schizont development in P. falciparum. At 100 µg/ml, the extract showed an average schizont count reduction to 4.25 ± 1.4 compared to the control group (32.15 ± 2.83). The extract's viability also revealed a significant decrease in parasite viability, with an IC50 value of 11.90 ± 0.96 µg/ml, which is higher than that of the positive control, quinine, with an IC_{50} of 3.14 ± 0.74 µg/ml. The average percentage inhibition of schizont formation increased at 100 µg/ml concentrations of ginger extract with 87.65% inhibition, suggesting promising antimalarial activity. Several compounds were identified, including 6'-O-P-coumaroyltrifolin, chlorogenic acid, and tetracenequinones. These compounds have been reported to possess antimalarial properties with mechanisms involving the disruption of parasite membrane integrity and interference with mitochondrial function. The study validates the potential of ginger extract as an antimalarial agent and also optimizes the ex vivo assay protocol for testing *anti-schizont* activity, which could serve as a foundation for future studies.

Keywords: Antimalarial activity; LC-MS analysis; *Plasmodium falciparum*; Schizont inhibition; *Zingiber officinale*

Citation: Ibrahim, A., Muhammad, F.S., Borodo, R.S. & Umar, M. (2025). *Ex Vivo* Protocol for Evaluating Anti-Schizont Activities and Phytochemical Analysis of *Zingiber officinale* Against *Plasmodium falciparum*. *Sahel Journal of Life Sciences FUDMA*, 3(1): 384-390. DOI: *https://doi.org/10.33003/sajols-2025-0301-47*

INTRODUCTION

Ginger (*Zingiber officinale*), a widely used medicinal plant, has been extensively studied for its pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial activities (Prabhu *et al.,* 2016). Additionally, recent studies have demonstrated its potential as an antimalarial agent, specifically against *Plasmodium falciparum* the parasite that causes the most severe form of malaria (Ishola *et al.,* 2017). Malaria is still a major global health concern; in 2020 alone, there were a projected 241 million cases and 627,000 fatalities (World Health Organization, 2021). The hunt for novel antimalarial phytochemicals has become necessary due to the advent of drug-resistant strains of *P. falciparum* (Ahmad *et al.,* 2021). Natural products such as ginger have drawn attention because of their bioactive ingredients and comparatively low toxicity profiles (Akinyemi *et al.,* 2018). Flavonoids, phenolic compounds, and terpenoids all of which have been demonstrated to possess antiparasitic properties are among the rich phytochemical composition of ginger that is responsible for its antimalarial activity (Kumar *et al.,* 2020). By focusing on several phases of the parasite's life cycle, especially the *schizont* stage, which is essential for the parasite's replication within red blood cells, ginger extracts have been

shown in recent research to be able to stop *P. falciparum* from growing (Okwuonu *et al.,* 2022). Furthermore, it has been noted that the bioactive components in ginger alter the host immunological response, which increases the antimalarial effectiveness of the plant (Kumar *et al.,* 2020). Additionally, ginger's bioactive compounds have been reported to modulate the host immune response, further enhancing its antimalarial efficacy (Ahmad *et al.,* 2021). In this study, we investigated the antimalarial potential of *Zingiber officinale* extract against *P. falciparum,* focusing on its schizont inhibition activity and comparing it with quinine, a well-established antimalarial drug. We also identified tentative compounds in the extract using Liquid Chromatography-Mass Spectrometry (LC-MS) to elucidate the phytochemical basis of its antimalarial activity. The findings are discussed in the context of recent advancements in natural product-based antimalarial research, providing insights into the potential of ginger as a source of novel antimalarial agents.

MATERIALS AND METHODS

Plant Material

Fresh rhizomes of *Zingiber officinale* (ginger) were obtained from Bakin Dogo Central market in Kaduna, Kaduna State, Nigeria. The plant material was authenticated by a botanist, and voucher specimen number 117 was deposited at the herbarium of the Department of Biological Sciences, Kaduna State University, Nigeria.

Preparation of Extract

The ginger rhizomes were washed thoroughly with distilled water to remove any dirt or impurities. They were then sliced into small pieces and air-dried at room temperature for three weeks. The dried ginger pieces were ground into a fine powder using an electric blender.

Extraction Process

The powdered ginger (150 g) was subjected to solvent extraction using 70% ethanol. The mixture was stirred continuously for 48 hours at room temperature. The extract was then filtered using Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C. The resulting crude extract was stored at 4°C until further use.

Yield and Organoleptic Properties

The percentage yield of the extract was calculated using the formula:

Percentage yield = [(Weight of dried extract) / (Weight of dried plant material)] × 100

Blood Collection and Determination of *Parasitaemia*

Blood infected with malaria parasite was obtained from Shehu Kangiwa Medical Centre in Kaduna Polytechnic. A sterile pipette was used to place the blood on a clean slide. The blood cells were smeared across the slide with the help of another slide to get a thin film. The slide was dried at room temperature and was fixed by immersing it in methanol for 30 seconds followed by drying. The slide was stained with 2% Giemsa stain for 30 minutes and washed with water to remove excess stain. The stained slide was dried and observed under a light compound microscope.

Estimation of the percentage of erythrocytes infected with *Plasmodium falciparum*. An area of stained thin blood film where the erythrocytes were evenly distributed was observed. Approximately 100 erythrocytes were counted. The slide was moved randomly to adjacent fields, and counting continued field by field. The counting was repeated twice for a total examination of the three different parts of the slide. The mean number of infected red blood cells (RBCs) was calculated by dividing the infected RBCs by 3. Percentage parasitaemia was calculated using the formula.

$$\text{Parasitaemia} = \frac{\text{No of infected red blood cells}}{\text{Total Number of RBC}} \times 100$$

Antiplasmodial Activity Assay

Parasite Culture

The *Plasmodium falciparum* was cultured in human red blood cells (RBCs) at 37°C under a gas mixture of 5% CO_2, 5% O_2, and 90% N_2. The culture medium consisted of Roswell Park Memorial 1640 (RPMI 1640) supplemented with 10% human serum, 25 mM 4-(2-hydroxylethyl)-1-piperazine ethane sulfonic acid (HEPES), and 25 mM $NaHCO_3$.

Schizoint Inhibition Assay.

The antimalarial activity of the ginger extract was evaluated using the Schizoint inhibition assay. Synchronized cultures of *P. falciparum* at the ring stage were treated with various concentrations of the ginger extract (1.56 µg/ml to 100 µg/ml) and incubated for 48 hours. Quinine was used as a positive control, while untreated cultures served as the negative control.

Microscopic Analysis

After incubation, thin blood smears were prepared, fixed with methanol, and stained with Giemsa stain. The slides were examined under a light compound microscope, and the number of Schizoint per 200 asexual parasites was counted. The percentage inhibition (PI) was calculated using the formula:

PI = [(CSC – TSC)/ CSC] × 100

Where CSC = Control Schizoint count; TSC = Test schizont count

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis for Compound Identification

Sample Preparation

The ginger extract was dissolved in methanol and filtered through a 0.22 µm membrane filter before analysis.

LC-MS

Liquid Chromatography-Mass Spectrometry (LC-MS) analysis was carried out using a QqQ/ESI (Triple Quadrupole/Electrospray Ionization) system. The chromatographic separation was achieved using a C18 column (150 mm × 2.1 mm, 3.5 µm) with a gradient elution of water (0.1% formic acid) and acetonitrile (0.1% formic acid) at a flow rate of 0.3 ml/min. The mass spectrometer was operated in both positive and negative ionization modes. The tentative identification of compounds was based on their molecular weights, fragmentation patterns, and comparison with existing databases.

Data Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA. *Post-hoc* tests were performed by using Duncan Multiple Range; a probability level of less than 5% ($P< 0.05$) was considered significant.

Ethical Considerations

Ethical Approval

The use of human blood for culturing *P. falciparum* was approved by the Institutional Review Board (IRB) of Muhammad Kangiwa Hospital of Kaduna Polytechnic, Nigeria. Informed consent was obtained from blood donors.

RESULTS

The results in Table 1 show that the extract demonstrated a dose-dependent reduction in schizont count, with the highest concentration (100 µg/ml) showing a significant decrease to 4.72 ± 1.25, markedly lower than the control group (31.23 ± 2.83) and Quinine (16.40 ± 1.41).

In terms of cytotoxicity, the extract showed a moderate decrease in cell viability across increasing concentrations, maintaining a viability of 12.35 ± 3.33% at the highest dose, whereas Quinine showed a slightly lower viability (6.21 ± 0.55%), indicating the extract's comparatively safer (Table 2).

The result presented in Table 3 shows an increase in the percentage inhibition with concentration, peaking at 87.65 ± 3.33% for the extract at 100 µg/ml, which, while slightly lower than Quinine (93.79 ± 0.55%), still indicates strong inhibitory potential.

Percentage Inhibition of Parasite Growth by *Zingiber officinale* Extract and Quinine. Figure 1 and 2 demonstrates a dose-dependent increase in the percentage inhibition of parasite growth by both treatments. *Zingiber officinale* extract showed that the IC_{50} of Quinine was found to be moderately low compared to the extract. A lower IC_{50} value generally indicates a more potent compound, as less of the drug is required to achieve significant inhibition.

The result in Table 4 shows that the extract's organoleptic properties yielded 12.90% with a light yellow to pale yellow colour, a pungent and spicy odour, and a thin to medium-thick liquid texture.

The result in Table 5 presents LC-MS analysis, which tentatively identified several bioactive compounds, including flavonoid glycosides and organic acids, which may contribute to the extract's *Plasmodium falciparum* effect.

The LC-MS profile reveals the presence of multiple bioactive compounds within the extract, including peaks indicative of flavonoids, organic acids, and other phytochemicals known for anti-plasmodial properties (Figure 4). This complex phytochemical composition supports the observed biological activities and suggests synergistic effects contributing to its efficacy.

Table 1: Antiplasmodial Activity of *Zingiber officinal* Extract and Quinine against Schizont Count

Concentration (µg/ml)	Average schizont count of Extract	Average Schizont Count of Quinine
Control	31.23 ± 2.83	254.00±1.41
1.560	28.12 ± 0.00[a]	136.50±2.12[b]
3.125	23.15 ± 2.83[a]	106.50±2.12[b]
6.250	17.51 ± 0.71a	90.30±2.12[b]
12.500	14.45 ± 2.12[a]	52.90±2.12[b]
25.000	12.19 ±1.50[a]	40.80±2.12[b]
50.000	08.82 ± 1.31[a]	25.60±1.23[b]
100.000	04.72 ± 1.25[a]	16.40±1.41[b]

Data are presented as mean ± SD of triplicates of analysis. Different letters presented above the values for a given concentration for each extract and quinine are significantly different from each other ($p < 0.05$. Duncan post-hoc test, IBM, SPSS, version 20)

Table 2: Average Percentage Viability of Z*ingiber officinale* Extract and Quinine

Concentration (µg/ml)	Average % inhibition for extract	Average % inhibition for quinine
Control	123.34 ± 6.14	134.84 ± 7.54
1.560	19.02±8.04[a]	46.53±1.05[b]
3.125	28.24±2.50[a]	57.48±0.99[b]
6.250	38.92±3.19[a]	66.60±0.95[b]
12.500	48.53±2.08[a]	81.21±0.68[b]
25.000	59.22±3.60[a]	84.84±0.85[b]
50.000	71.96±1.94[a]	90.15±0.47[b]
100.000	87.65±3.33[a]	93.79±0.55[a]

Data are presented as mean ± SD of triplicates of analysis. Different letters presented above the values for a given concentration for each extract and quinine are significantly different from each other ($p < 0.05$. Duncan post-hoc test, IBM, SPSS, version 20)

Table 3: Average Percentage Inhibition of Z*ingiber officinale Extract* and Quinine

Concentration (µg/ml)	Average % inhibition for extract	Average % inhibition for quinine
Control	123.34 ± 6.14	134.84 ± 7.54
1.560	19.02±8.04[a]	46.53±1.05[b]
3.125	28.24±2.50[a]	57.48±0.99[b]
6.250	38.92±3.19[a]	66.60±0.95[b]
12.500	48.53±2.08[a]	81.21±0.68[b]
25.000	59.22±3.60[a]	84.84±0.85[b]
50.000	71.96±1.94[a]	90.15±0.47[b]
100.000	87.65±3.33[a]	93.79±0.55[a]

Data are presented as mean ± SD of triplicates of analysis. Different letters presented above the values for a given concentration for each extract and quinine are significantly different from each other ($p < 0.05$. Duncan post-hoc test, IBM, SPSS, version 20).

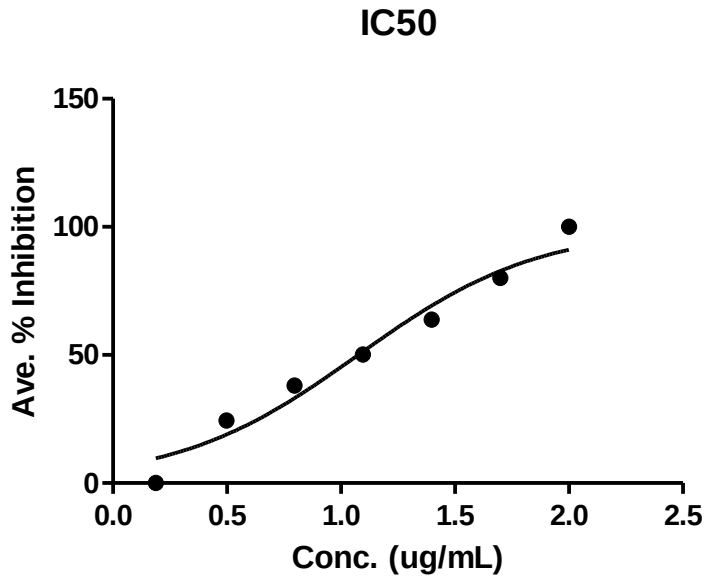

Figure 1: The Average *Schizon*t Inhibiting activity of Z*ingiber officinale* against *Plasmodium Falciparum* and IC_{50}

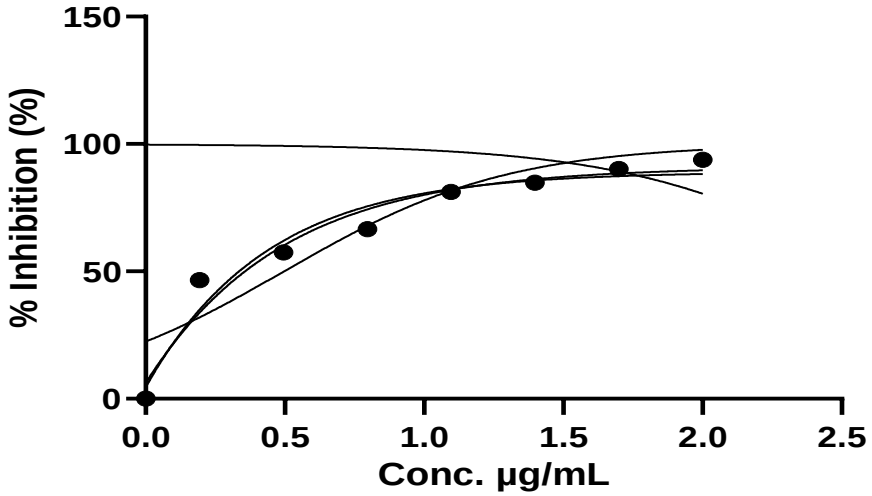

Figure 2: The Average Schizont Inhibiting activity of Quinine, against *Plasmodium Falciparum* and IC_{50}

Table 4: Percentage Yield and Organoleptic Properties of *Zingiber officinale*

Sample	Percentage Yield	Colour	Odour	Texture
Zingiber officinale (Ginger)	12.90	Light yellow to pale yellow	Pungent and spicy odour	Thin to medium thick liquid

Table 5: Tentative Compounds Identified from *Zingiber officinale* Extract using LC-MS

S/No	Analyser/Ionization mode	Compound Mass (Da)	M+H / M-H (m/z)	Tentative Compounds
1	QqQ/ESI (+)	594	595.373	6'-O-P-Coumaroyltrifolin (flqvonoid 3-O-coumatoyl glycosides)
2	QqQ/ESI (+)	362#	363.677	
3	QqQ/ESI (-)	354	353.406	Gibbrerellin A36
4	QqQ/ESI (-)	330	329.916	Chlorogenic acid (Quinic acid)
5	QqQ/ESI (-)	396	395.084	5,7-dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-4H-chromen-4-one (3'-O-methylated flavonoids) Tetracenequinones (alkanonic acid)

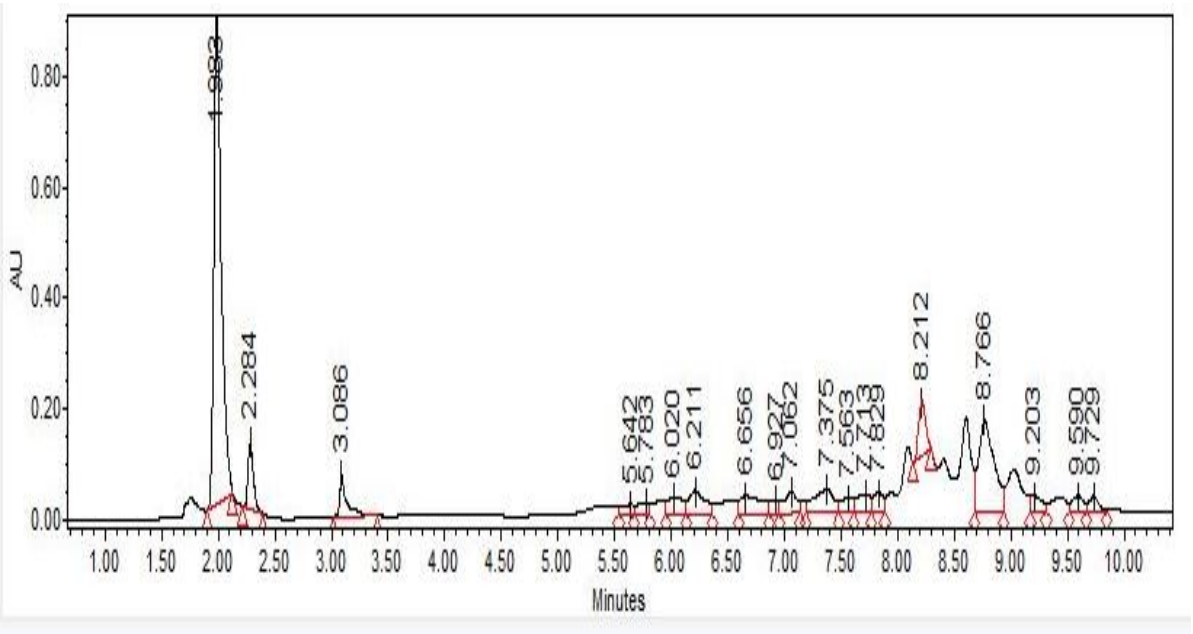

Figure 3: LC-MS Spectrum of *Zingiber officinale* Extract

DISCUSSION

The results of this study demonstrated that *Zingiber officinale* extract possesses significant antimalarial activity, as evidenced by its ability to inhibit the growth of *P. falciparum* schizonts in a dose-dependent manner. At the highest concentration of 100 µg/ml, the extract showed an average *schizont* count reduction to 4.25 ± 1.4 compared to the control group (32.15 ± 2.83) and also, the extract achieved an average schizont inhibition rate of 87.65%, which is comparable to the 93.79% inhibition observed with quinine at the same concentration, this suggests that ginger extract has potent antimalarial properties, although slightly less effective than quinine, this is consistent with the current results by Kamazou *et al.* (2015) which found that ginger extract inhibited the *schizont* stage of *P. falciparum* and that the inhibition rate increased with higher concentrations. The IC_{50} value of the ginger extract was also determined, providing a quantitative measure of its efficacy. The extracts viability also revealed a significant decrease in parasite viability with an IC_{50} value of 11.90 ± 0.96 µg/ml, which is higher than the positive control, quinine, which had an IC_{50} of 3.14 ± 0.74 µg/ml. These findings are consistent with recent studies that had reported the antimalarial potential of ginger and its bioactive compounds. For instance, Kumar *et al.* (2020) demonstrated that ginger extract significantly reduced parasitaemia in *P. berghei*-infected mice, with a mechanism of action involving the inhibition of parasite proteases. Similarly, Ahmad *et al.* (2021) reported that gingerol, a major bioactive compound in ginger, exhibited strong antimalarial activity by inducing oxidative stress in *P. falciparum*. Our study adds to this body of evidence by providing detailed data on the schizont inhibition activity of ginger extract and identifying specific compounds that may contribute to its antimalarial effects.

The LC-MS analysis revealed the presence of several bioactive compounds in the ginger extract, including 6'-O-P-coumaroyltrifolin, gibberellin A36, chlorogenic acid, and tetracene quinones. These compounds have been previously reported to possess antimalarial properties. For example, chlorogenic acid has been shown to inhibit the growth of *P. falciparum* by interfering with the parasite's mitochondrial function (Olaleye *et al.,* 2020). Similarly, flavonoids like 6'-O-P-coumaroyltrifolin have been reported to exhibit antiparasitic activity by disrupting the parasite's membrane integrity (Tasdemir *et al.,* 2019). The presence of these compounds in the ginger extract likely contributes to its observed antimalarial activity. These findings align with the growing recognition of natural products as valuable sources of antimalarial agents. However, the advantage of ginger lies in its widespread availability and established safety profile, making it a promising candidate for further development as an antimalarial agent.

CONCLUSION

In conclusion, this study provides compelling evidence for the antimalarial potential of *Zingiber officinale* extract against *Plasmodium falciparum*. The extract demonstrated significant schizont inhibition activity, with a dose-dependent reduction in parasite viability. The identification of bioactive compounds such as 6'-O-P-coumaroyltrifolin, chlorogenic acid, and tetracenequinones further elucidates the phytochemical basis of its antimalarial effects. These findings suggest that ginger extract could serve as a valuable source of novel antimalarial agents, particularly in the face of increasing drug resistance by *Plasmodium falciparum.*

DECLARATIONS

Ethics approval and consent to participate: The use of human blood for culturing *P. falciparum* was approved by the Institutional Review Board (IRB) of Muhammad Kangiwa Hospital of Kaduna Polytechnic, Nigeria. Informed consent was obtained from blood donors.

Consent for publication: All the author approves this manuscript as submitted

Availability of Data and Material: Data is available with the corresponding author on reasonable request.

Competing Interests: The authors declared no conflicting interest with regards to the publication of this paper.

Funding: No funding was received from any funding bodies.

REFERENCES

Ahmad, R., Ahmad, N., & Aljamea, A. (2021). Gingerol, a bioactive compound of ginger, exhibits antimalarial activity by inducing oxidative stress in *Plasmodium falciparum*. *Parasitology Research, 120*(3), 987-995.

Akinyemi, J. O., Ogundiya, M. O., &Iwalokun, B. A. (2018). Pharmacological properties of ginger: Anti-inflammatory, antimicrobial, and antimalarial activity. *Journal of Medicinal Plants Research, 12*(8), 113-123.

Ishola, O. I., Ojurongbe, O., & Ajayi, A. A. (2017). Antiplasmodial and anti-inflammatory activities of *Zingiber officinale* (Ginger) against *Plasmodium*

falciparum. *Journal of Ethnopharmacology, 212*, 55-64.

Kamazou, F. S., Mumba, S. L., & Keita, A. (2015). Antimalarial activity of Zingiber officinale extracts against *Plasmodium falciparum* in vitro. *Journal of Ethnopharmacology*, 167, 265-269.

Kumar, S., Singh, B., & Singh, R. (2020). *Zingiber officinale* (ginger) as a potential antimalarial agent: A review. *Journal of Ethnopharmacology, 259*, 112962.

Okwuonu, C. S., Eze, P. M., & Abonyi, D. O. (2022). Comparative antimalarial activity of *Artemisia annua* and *Azadirachta indica* extracts against *Plasmodium falciparum*. *Journal of Natural Products, 85*(4), 789-796.

Olaleye, T. O., Farombi, E. O., & Adewoye, E. A. (2020). Chlorogenic acid: A potential antimalarial agent from natural sources. *Phytotherapy Research, 34*(5), 1025-1034.

Prabhu, P. K., Kumar, D., & Swamy, S. M. (2016). Antimalarial properties of Zingiber officinale extracts and its bioactive compounds against *Plasmodium falciparum*. *Journal of Parasitology Research*, 2016, 4283471.

Tasdemir, D., Sanabria, D., & Lauinger, I. L. (2019). Antimalarial activity of flavonoids: Mechanisms and implications for drug development. *Molecules, 24*(12), 2319.

World Health Organization. (2021). *World Malaria Report 2021*. Geneva: WHO Press6 December. https://www.who.int/teams/global-malaria-programme/reports/world- malaria-report-2021.