In-vivo anti-plasmodia activity of *Enantia chlorantha:* A potential *Plasmodium* berghei transketolase inhibitor.

ABSTRACT

Introduction: The emergence of resistance to existing antimalarial drugs has intensified the search for novel, effective, and affordable treatments for malaria. Deactivating transketolase provides a unique approach to disrupting parasite survival and addressing the rising drug resistance in malaria treatment. Enantia chlorantha, a plant species traditionally used in African folk medicine, has shown promise as a potential antimalarial agent by inhibiting transketolase activity. This study aimed to investigate the antimalarial efficacy of *Enantia chlorantha* extracts in a murine model of malaria using in vivo approach.

Method: A total of 55 albino mice inoculated with an infection of 1.0×10^7 chloroquine resistance strain of *Plasmodium berghei* intra-peritoneally. The level of parasitaemia and death per day were used to assess the chemosuppressive effect and survival rate of the extracts. Experimental mice were treated for four days administering orally a dose of 5mg/kg, 10mg/kg, and 20mg/kg of each plant extract and a dose of 10mg/kg chloroquine was used as standard control drug. Data were analyzed using One Way Analysis of variance ANOVA followed by post hoc test, and were expressed as mean ± standard error of mean (M ± SEM), and percentage. All analyses were carried out at a 95% confidence interval, with a significance level set at $P < 0.05$.

Results: The treatment with various concentrations of *Enantia chlorantha* extracts (EC2, EEA, and EHEX) significantly reduced parasitaemia levels, with higher doses showing greater suppression. EC2 reduced parasitaemia from 1.00% (5 mg/kg) to 0.71% (20 mg/kg) with p=0.0003, while EEA reduced it from 1.15% (5 mg/kg) to 0.57% (20 mg/kg) with p=0.0028. EHEX showed a suppression from 1.09% (10 mg/kg) to 0.46% (20 mg/kg) with p=0.0001. Chemosuppression in EC2, EEA, and EHEX increased with dosage, with EEA at 20 mg/kg surpassing chloroquine in chemosuppression (85.64% vs. 69.86%, p=0.0009). In survival outcomes, EC2, EEA, and EHEX exhibited dose-dependent increases in survival index, with EEA at 20 mg/kg reaching 90%, but none matched chloroquine's 100% survival,

Conclusion: *Enantia chlorantha* extracts particularly EEA demonstrated antimalarial effects at high doses and could be potential transkelotase inhibitors, as they significantly reduced parasitaemia level, increased chemosuppressive effect and survival index.

Keywords: Enantia chlorantha, Malaria, Plasmodium berghei,* Parasitaemia, *Chemosuppression, transketolase.

INTRODUCTION

Malaria remains a critical global health challenge affecting tropical and subtropical regions where it contributes to morbidity and mortality. Approximately 619,000 deaths in 247 million cases of malaria have been recently recorded, with the highest burden occurring in sub-Saharan Africa (WHO, 2021). Children under five years of age and pregnant women remain the most populations vulnerable to malaria. Despite the

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advancements in prevention and treatment strategies for the control of malaria, the emergence and spread of drug-resistant strains of *Plasmodium falciparum* remains a substantial challenge (Wilson et al., 2020). The compromise in the effectiveness of several existing antimalarial drugs shows the development of resistance by malaria parasites particularly *Plasmodium falciparum*. Chloroquine, being the gold standard for malaria treatment, lost efficacy due to mutations in its 'resistance transporter' gene (*PfCRT)*, which lowered the parasite's susceptibility to the drug (Ansbro, 2020). Likewise, the deactivation of dihydrofolate reductase and dihydropteroate synthase as a result of mutations of the enzymes' genes, has render antifolate drugs such as sulfadoxine-pyrimethamine (SP) less effective in eradicating malaria parasite (Rout et al., 2020). Artemisinin-based combination therapies (ACTs), currently the most effective treatment for uncomplicated malaria, are also under threat. Mutations in the *Pfkelch13* gene have been reported particularly in Southeast Asia and sub-Saharan Africa to cause delay in parasite clearance in the blood (Wangai et al., 2020; Milong et al., 2024). These developments underscore the urgent need for novel antimalarial drugs and innovative strategies to combat resistance.

Targeting essential metabolic pathways in *Plasmodium* offers a promising approach for developing new treatments. The pentose phosphate pathway (PPP) is a crucial pathway in nucleotide synthesis and redox balance, which are essential for the growth and survival of malaria parasites (Jezewski et al., 2021). Transketolase, a key enzyme in the PPP, facilitates the interconversion of sugar phosphates necessary for nucleic acid and amino acid synthesis (Ali et al., 2024). Studies have shown that inhibiting transketolase activity significantly impairs *Plasmodium falciparum* growth, making it an attractive target for antimalarial drug development (Boateng et al., 2020; Morales-Luna et al., 2024; Alkurbi et al., 2024). Natural products derived from medicinal plants have historically served as a rich source of antimalarial compounds. Traditional medicine continues to play a vital role in healthcare, especially in resource-limited settings. About 80% of the population in developing countries relies on traditional medicine for primary healthcare needs (Kebede et al., 2021). Among these medicinal plants, *Enantia chlorantha*, commonly referred to as "Awopa" in western parts of Nigeria, holds significant potential for addressing malaria and other ailments. Widely used in folk medicine, *Enantia chlorantha* has demonstrated therapeutic efficacy against conditions, including malaria, aches, wounds, yellow fever, hepatitis, typhoid fever, leprosy spots, tuberculosis, and various gastrointestinal disorders, among others. Additionally, it is used as a haemostatic agent and uterine stimulant (Olowo et al., 2022). Phytochemical research has identified bioactive compounds in *Enantia chlorantha*, such as alkaloids, flavonoids, and saponins, which are known to possess antiplasmodial, anti-inflammatory, and antimicrobial properties (Abubakar et al., 2020; Akinwale et al., 2022). Despite its traditional use and recognized antiplasmodial activity, there is a significant gap in understanding the specific mechanisms through which *Enantia chlorantha* exerts its effects. Recent studies suggest that its bioactive compounds may act by inhibiting transketolase, thereby disrupting the PPP and impairing parasite survival (Ghani et al., 2024; Morales-Luna et al., 2024). Inhibiting transketolase activity significantly impairs the growth of *Plasmodium spp*, making it a promising target

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for new antimalarial treatments. Natural extracts, particularly from *Anacardium occidentale* (cashew), have been found to exhibit antiplasmodial activity by targeting transketolase, as demonstrated by Kaushik et al. (2023). This study therefore aimed to investigate the antimalarial efficacy of *Enantia chlorantha* extracts (berberin alkaloid, ethyl acetate and n-hexane) in inhibiting of *P. berghei* transketolase using in vivo approach.

MATERIALS AND METHOD

Materials and chemicals

Chloroquine (IPCA Pharmaceuticals Nig. Ltd), Virgin Olive oil, distilled water, normal saline needles and syringes, animal feeds, cages, light microscope, microscope glass slide, Chloroform, extractor, Giemsa stain

Experimental Design

This *in-vivo* experiment involved chemosuppressive tests and survival index assessments on *Plasmodium berghei*-infected mice according to the method of Knight and Peters, (1980) and Onyeto et al. (2019).

Animal Grouping

- A total of 55 Adult albino mice (30 female and 25 male) of weight 18-23g were obtained from the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The mice were acclimatized for five days to laboratory conditions prior to the experiment. They were allowed free access to food and water *ad libitum* throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces from cages daily. The mice were maintained and cared for according to the international guidelines for the use and maintenance of experimental animals (OECD, 2002).

    - The mice were randomized into 5 groups of 11 mice as follows:

      Group 1: *Plasmodium berghei* infected mice treated with varying concentration (5mg/kg, 10mg/kg, and 20mg/kg) methanol extract of *Enantia chlorantha* [EC2] on parasitaemia level.

      Group 2: *Plasmodium berghei* infected mice treated with varying concentration (5mg/kg, 10mg/kg, and 20mg/kg) of ethyl acetate extract of *Enantia chlorantha* [EEA] on parasitaemia level.

      Group 3: *Plasmodium berghei* infected mice treated with varying concentration (5mg/kg, 10mg/kg, and 20mg/kg) of n-hexane extract (EHEX) of *Enantia chlorantha* [EEA] on parasitaemia level.

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Group 4: (**Positive control)** *Plasmodium berghei* infected mice treated with varying concentration with varying concentration (5mg/kg, 10mg/kg, and 20mg/kg) of gold standard drug-'**Chloroquine'** on parasitaemia level.

Group 5: (**Negative control)** *Plasmodium berghei* infected mice treated with varying concentration with varying concentration (5mg/kg, 10mg/kg, and 20mg/kg) of '**Olive oil'** on parasitaemia level.

Parasite

Chloroquine-sensitive *Plasmodium berghei* NK-65 was obtained from the Institute of Advanced Medical Research and Training (IMRAT), College Hospital, Ibadan, University of Ibadan.

Inoculation of the mice

Each mouse was inoculated with 0.2 ml of parasitized blood containing 1.0 x 10^7 chloroquine resistance strain of *Plasmodium berghei* intra-peritoneally for 1 day, and treated for 4 days. On the 5^{th} day post-treatment, parasitaemia levels were monitored in each group for 4 days and were compared to both positive and negative controls.

Ethical Approval

Ethical Approval was obtained from the Ethical Research Committee of the Faculty of Basic Medical Sciences, LAUTECH, Ogbomoso. Ethical Research Committee Approval Number**:** ERCFBMSLAUTECH: 083/11/2024.

Plant Parts Collection and Authentication

The stem-bark of *Enantia chlorantha* was purchased from Ethnomedicine store at Ojagbo market, Ogbomoso, Oyo State, Nigeria and was identified by botanists at the department of plant biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria for authentication and the voucher specimen was deposited at the herbarium of the University with the ***voucher number LHO 878***.

Plant Preparation and Extraction

The stem-bark was crushed into small piece and 587.98g of the crushed stem bark which was loaded into the Sohxlet extractor and extracted with 500ml of methanol, n-hexane and ethyl acetate successively. After each extraction, the solvent was removed under reduced pressure using a rotary evaporator to obtain a concentrated extract of *Enantia chlorantha* stem-bark. Each concentrated extract was stored in airtight containers at room temperature until further analysis and experimental use. The methanol extract was blended with silica gel and packed on a column with silica gel for chromatographic separation and concentrated using the rotary evaporator to obtain pure compounds which were recrystallized from suitable solvents.

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Sample Collection

After the 4th day of treatment, the mice were euthanized using chloroform and 5mls of blood was collected from each mouse via cardiac puncture into heparinized bottle. The red blood cell per unit volume was calculated for the inoculums size. The desired volume of blood then obtained from the donor mouse was suitably diluted with sterile normal saline so that the final (0.2 ml) for each mouse would contain the required number of red blood cells ($1.0x10^7$ parasitized red blood cells).

Phytochemical Analysis of the Extracts

Phytochemical analysis of the n-hexane and ethyl acetate extract of *E. Chlorantha* stem bark was performed using Agilent GC-7890A series Gas Chromatograph. Identification of volatiles components was based on GC retention indexes calculated by mass spectra and computerized matching of compounds with the National Institute of Standards and Technology (NIST), Timberland Regional Library (TRLIB) and Wiley libraries as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature.

Suppressive Assays

i. Parasitaemia and Chemosuppression levels [Knight and Peters, 1980]

Thin smear of each mouse was done by taking a drop of blood from a tiny tail cut on a slide and spreading thinly with another slide. The smear was air-dry, and then fixed with methanol. The smear was then stained with prepared Giemsa stain (diluted 1:20 with phosphate buffered saline), placed on the light microscope and viewed under oil immersion objective(X100). The total number of parasitized red blood cells and red blood cells was counted in ten fields. The Percentage parasitaemia and chemosuppression in each field was calculated as shown below:

a. Percentage parasitaemia in each field was calculated as follows:

$$\%P = \frac{\sum PRBC}{\sum RBC} \times 100$$

Where *P* = Parasitaemia,
PRBC = parasitized red blood cells
RBC = red blood cells.

b. Calculation of average percentage chemosuppression or reduction in parasitaemia:

$$\%CM = \frac{\%P_{Negativecontrol} - \%P_{Testgroup}}{\%P_{Negativecontrol}} \times 100$$

Where *CM* = chemosuppression
P = parasitaemia

ii. Survival index

The survival index was estimated based on the observed day of death of the animals in each test group including the negative control. The animals were observed for 28 days post infection

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and the day of death recorded for each animal. The formula below was used to calculate the survival index.

$$SI = \frac{D_{Test} - D_{Negativecontrol}}{D_{Max} - D_{Negativecontrol}} \times 100$$

Where *SI* = Survival index
D_{Test} = average day of Death for test group
D_{Max} = Maximum observation day which is 28 for this study and
$D_{Negative\ control}$ = average day of Death for negative control group.

Method of Statistical Analysis

Data were analyzed using One Way Analysis of variance ANOVA followed by *Post hoc* test, and were expressed as mean ± standard error of mean (M ± SEM) for central tendency and variability of the results across test and control groups. All analyses were carried out at a 95% confidence interval, with a significance level set at $P < 0.05$. Percentage parasitaemia, chemosuppression, and survival index were calculated and expressed with Bar-charts to quantify the treatment effects.

RESULTS

1. Phytochemical Results

The Phytochemical analysis of *E. Chlorantha* extracts revealed 51 bioactive compounds as shown in table 1 below.

Table 1 The Bioactive compounds isolated from *E. Chlorantha* extracts

Isolated Bioactive compounds of *E. Chlorantha*	
1. Docosene 2. Berberine 3. 1-methylbicyclo(3.2.1)octane 4. 1-nonadecene 5. 10,10-dimethyl-2,6-dimthylenebicylco(7.2.0)-decane 6. 17-pentatriacontene 7. 1H-Benzocyclohepten-7-ol,2,3,4,4a,5,6,7,8-octahydo-1,1,4a,7-tetramethyl-cis 8. 1H-cycloprop(e)azulene 9. 2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-napthalen-2-yl)-prop-2-en-1-ol 10. 2-Adamantylamine, N-acetyl 11. 2-pyrrolidinone 12. 4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo(4.1.0)heptane 13. 9-Octadecenoic acid (Z)-, methyl ester 14. 9-Octadecenoic acid (Z) 15. 9-Octadecenoic acid	27. Methyl Stearate 28. Naphthalene 29. Octadecanoic acid 30. Oxalic acid 31. Tetracosane 32. Tridecanoic-acid 33. Undecanoic acid 34. Vaccenic-acid_-cis 35. Z-5-Nonadecene 36. bicyclo 5.2.0 nonane 37. caryophyllene oxide 38. Cyclohexene 39. hexadecanoic acid, methyl ester 40. n-Hexadecanoic acid 41. Nonadecane 42. pentacos-1-ene 43. trans-13-Octadecenoic-acid 44. Aporphine 45. Berberine

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16. Alloaromadendrene oxide 17. Aromadendrene oxide 2 18. Benzoic acid 19. Bicyclo[2.2.1]heptane, 1,3,3-trimethyl-, (1S,4R)- 20. Bis(2-ethylhexyl) phthalate 21. Cyclotetracosane 22. Dodecanoic acid 23. Eicosane 24. Hexadecane 25. Hexadecanoic acid 26. Isopropyl Palmitate	46. Columbamin 47. Isoquinoline 48. Jahorrtizine 49. Palmatine 50. Phenanthrene 51. Protoberberin

2. Level of Parasitaemia after 4 Days of Drug Administration

This finding shows the parasitaemia level after the treatment with various concentration of *Enantia chlorantha* extracts (EC2, EEA, and EHEX) with significant suppression seen at higher doses.

Parasitaemia level in **EC2** treated group significantly reduced from 1.00% (SEM±0.20) at 5mg/kg to 0.80% (SEM±0.06) at 10 mg/kg, and further to 0.71% (SEM±0.13) at 20 mg/kg with p=0.0003.

Parasitaemia level was also suppressed from 1.15% (SEM±0.29) at 5mg/kg to 0.92% (SEM±0.10) at 10mg/kg and then to 0.57% (SEM±0.08) at 20mg/kg with p= 0.0028 in **EEA** treated group.

However, parasitaemia level significantly suppressed only from 1.09% (SEM±0.26) at 10mg/kg to 0.46% (SEM±0.12) at 20mg/kg with p= 0.0001. Although effective, EHEX displayed slightly lower suppression levels compared to EC2 and EEA.

The positive control group treated with **chloroquine** at 10 mg/kg showed a parasitaemia level of 0.97% (SEM ± 0.23) when compared to EC2 and EEA treatment group (p=0.0009).

The negative control group treated with **olive oil** at 10 mg/kg displayed a much higher parasitaemia level of 3.21% (SEM ± 0.54) with p=0.0001.

Table 2: Percentage Parasitaemia after 4 Days of Drug Administration

Groups	**Concentration** (mg/kg)	**% Parasitaemia**	**Mean±SEM**	**P.Value**
Group 1: EC2 treated	5	1.00	1.00±0.20	0.0003*
	10	0.80	0.80±0.06	
	20	0.71	0.71±0.13	
Group 2: EEA treated	5	1.15	1.15 ±0.29	0.0028*
	10	0.92	0.92±0.10	
	20	0.57	0.57±0.08	

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Group 3: EHEX treated	5	2.23	2.23±0.73	0.0001*
	10	1.09	1.09±0.26	
	20	0.46	0.46±0.12	
Group 4: CQ 10 treated	10	0.97	0.97±0.23	0.0009 *
Group 5: O/O treated	10	3.21	3.21±0.54	0.0001*

Data were presented as mean ± standard error of mean (SEM)

** = statistically significant*

KEY: CQ - chloroquine (Positive control)

O/O – olive oil (Negative control)

2. Chemosuppressive Activity after 4 Days of Drug Administration

The percentage Chemosuppression observed in EC2 treatments group increased with dosage. At a dose of 5 mg/kg, EC2 demonstrated a chemosuppression of 68.67%, this was further enhanced at higher dosage of 10mg/kg to 75.08% and at 20mg/kg reaching 77.69%. EC2 showed notably the same chemosuppression rate with the positive control at 5mg/kg.

Chemosuppressive effect of EEA is dose-dependent. At 5 mg/kg, EEA achieved a 21.15% chemosuppression rate, indicating initial efficacy at a low dose. This effect markedly increased at 10 mg/kg, where EEA reached 73.24% suppression, and further escalated to an impressive 85.64% at 20 mg/kg. EEA demonstrated highly effective antimalarial properties at 10 mg/kg and at 20 mg/kg than the positive control with a suppression rate of 69.86%,

Similarly, the chemosuppressive effect of EHEX treatment increased as dose increases. At a concentration of 5 mg/kg, EHEX achieved a 63.99% chemosuppression rate which rose to 71.34% at 10 mg/kg and further increased to 82.13% at the highest dose of 20 mg/kg. This result also indicates that EHEX is effective in reducing parasitaemia in a dose-dependent manner. When compared to the positive control with a suppression rate of 69.86%, EHEX demonstrated comparable efficacy at 10 mg/kg and surpassed it at 20 mg/kg.

In comparison with the positive control with a suppression rate the extracts of *Enantia chlorantha* shows a strong dose-response, with increased concentrations correlating to increased parasitaemia inhibition.

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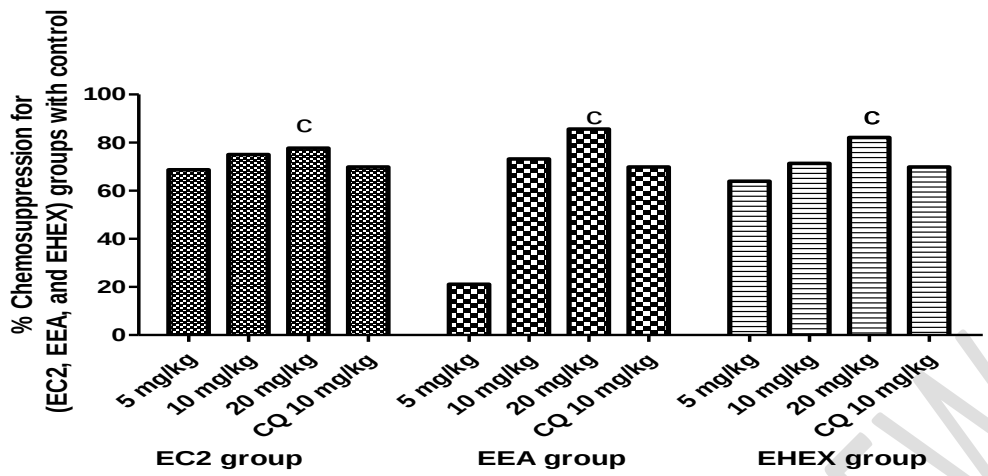

Figure 1: Effect of Varying Dosage of EC2, EEA, and EHEX on %Chemosuppression.
Chemosuppression rate is expressed in %, with c= highest chemosuppression rate.

3. Survival Index

Table 3 shows the average day of death across groups varying dosage of EC2, EEA, and EHEX. The results shows no significant differences in average day of death in groups treated with varying dosage of EC2, EEA, and EHEX, but significantly reduced compared with the positive control at CQ10mg/kg with p= 0.0413.

Figure 2 shows that survival index in infected mice after treatment with Varying Dosage of EC2, EEA, and EHEX and CQ is dose-dependent. Treatment with 5, 10, and 20 mg/kg of EC2 showed an increasing survival index of 63.6%, 64.5%, and 72.7% respectively. Also, 5, 10, and 20 mg/kg of EEA shows an increasing survival index of 9.1%, 81%, and 90% respectively, while 5, 10, and 20 mg/kg of EHEX shows an increasing survival index of 36.4%, 45.5%, and 45.5% respectively.

The survival index in infected mice after treatment with CQ was 100%, highlighting chloroquine's superior survival-sustaining effect in infected mice. These results demonstrate that while EC2, EEA, and EHEX contributed to moderate survival, none matched the significant efficacy of chloroquine.

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Table 3: The table presents the average death across all group over a period of 28 days

	Conc (mg/kg)	AVG day of Death	SEM	P Value
EC2 Test group A	5	23.75	23.75 ±2.46	0.7456
	10	22.0	22.0 ±3.18	
	20	24.75	24.75 ± 2.36	
EEA Test group B	5	17.25	17.25 ±1.44	0.999
	10	26.25	26.25 ±1.75	
	20	25.50	25.50 ±2. 50	
EHEX Test group C	5	20.75	20.75 ±1.49	
	10	21.75	21.75 ±3.07	0.9646
	20	21.50	21.50 ±2.25	
Control Groups	CQ 10	28.00	28.00±0.00	0.0413*
	O/O	17.00	17.00±5.00	-

Data were presented as mean ± standard error of mean (SEM)

** = statistically significant*

KEY: CQ - chloroquine (Positive control)
O/O – olive oil (Negative control)

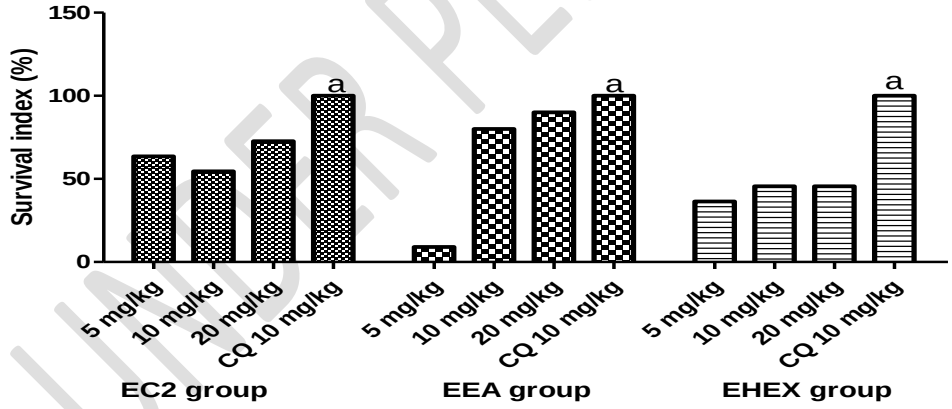

Figure 2: Survival index of varying dosage of EC2, EEA, and EHEX compared to CQ on infected mice. Survival index is expressed in %, with a= highest Survival index.

4. Comparative Analysis Chemosuppression and Survival Index for EC2, EEA, and EHEX with Positive Control

A comparative analysis of EC2, EEA, and EHEX reveals varying levels of efficacy. EEA and EHEX at 20 mg/kg outperformed Chloroquine in chemosuppression, establishing them as the most potent antimalarial. EC2 and EHEX also showed strong antimalarial

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effects, with chemosuppressive and survival rates increasing significantly at higher doses, though slightly lower than EEA. Chloroquine, the positive control, served as a benchmark; however, EEA extract frequently matched or exceeded its effectiveness, suggesting that these natural compounds could potentially serve as alternatives to or in combination with traditional antimalarials.

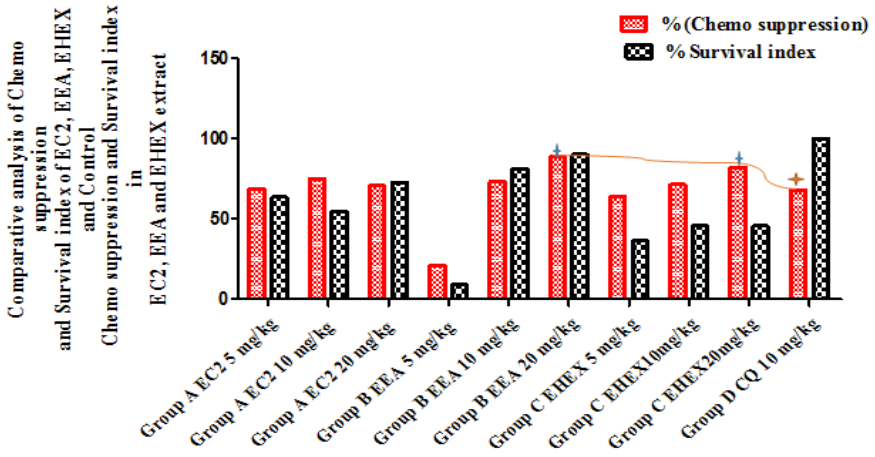

Figure 3: Comparative analysis of Chemo suppression and Survival index for EC2, EEA, EHEX and positive control. Blue star= higher chemosuppressive effect than CQ. Red star= chemosuppression level of CQ.

DISCUSSION

Malaria constantly remains the most critical public health challenges worldwide, especially in tropical and subtropical regions (Kolawole et al., 2023). The emergence of drug-resistant *Plasmodium* species has complicated the treatment landscape, highlighting the urgent need for new and effective antimalarial agents (Siqueira-Neto et al., 2023). Among the many potential sources of novel therapies, plants have long been a cornerstone of antimalarial drug discovery. *Enantia chlorantha*, a medicinal plant used in traditional medicine across Africa, has shown promise in various studies for its potential antimalarial properties (Abubakar et al., 2020; Akinwale et al., 2022). This study evaluates the effects of different extracts from *Enantia chlorantha* EC2, EEA, and EHEX on parasitaemia levels in mice infected with *Plasmodium*. By comparing the results with the positive control chloroquine and a negative control olive oil, the findings highlight the potential of these extracts as viable alternatives or complements to conventional malaria treatments.

Parasitaemia, or the presence of parasites in the bloodstream, is a key indicator of the severity of malaria and the effectiveness of treatment (White, 2021). A finding of this study revealed that all three extracts of *Enantia chlorantha* (EC2, EEA, and EHEX) significantly reduced parasitaemia in a dose-dependent manner, which indicates their potential as effective antimalarial agents. Their antiplasmodial activity could either be rendered via oxidative lysis of *P. berghei* or by inhibiting transkelotase (Onyeto et al.,

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2019). In the case of EC2, parasitaemia levels were observed to decrease significantly with higher doses. At a dose of 5 mg/kg, the parasitaemia level was reduced to 1.00%, and at 10 mg/kg, it further dropped to 0.80%. The most substantial reduction occurred at 20 mg/kg, where parasitaemia levels were suppressed to 0.71%. These results suggest that EC2 is effective at reducing parasitic load, with increasing doses leading to more pronounced suppression (Ibrahim, 2019).

EEA exhibited even stronger antimalarial effects than EC2. At a dose of 5 mg/kg, parasitaemia was reduced to 1.15%, which is comparable to EC2's performance. However, at 10 mg/kg, the parasitaemia level decreased further to 0.92%, and at the highest dose of 20 mg/kg, parasitaemia was significantly reduced to 0.57%. This indicates that EEA may contain more potent bioactive compounds that effectively combat *Plasmodium* infection, especially at higher doses (Babalola et al., 2020). EHEX, although effective, showed slightly lower suppression levels than both EC2 and EEA. This result shows that while EHEX was still effective, its potency at moderate doses was not as strong as EEA or EC2. Nonetheless, EHEX demonstrated the ability to reduce parasitaemia significantly, particularly at the highest dose of 20 mg/kg. In comparison with the positive control, chloroquine, which reduced parasitaemia to 0.97% at 10 mg/kg, both EC2 and EEA showed comparable or even superior suppression at higher doses (Ezenyi et al., 2020). In contrast, the negative control group, which received olive oil at 10 mg/kg, displayed much higher parasitaemia levels (3.21%), reinforcing the effectiveness of the extracts in reducing the parasitic load in infected mice.

Chemosuppression, or the percentage reduction in parasitaemia, is another important indicator of an antimalarial agent's effectiveness (Oyinloye et al., 2024). This study revealed a clear dose-dependent increase in the chemosuppressive effects of the *Enantia chlorantha* extracts. Chloroquine, a well-established antimalarial drug, served as the positive control in this study. At a dose of 10 mg/kg, chloroquine reduced parasitaemia to 0.97%, which is consistent with the findings of previous studies that highlight chloroquine's effectiveness in malaria treatment. EEA exhibited the most potent chemosuppression among the three extracts and the most significant suppression occurred at 20 mg/kg, with a chemosuppression rate of 85.64%. At this dose, EEA's chemosuppression surpassed the 69.86% observed with chloroquine, suggesting that EEA may have a comparable or even superior effect at higher doses. EC2 and EHEX also displayed significant chemosuppression, although not as strong as EEA. These results highlight that EC2 and EHEX are also potent antimalarial agents, especially at higher doses, with its efficacy surpassing chloroquine at the highest dose of 20 mg/kg. in agreement with other studies, these findings suggest that *Enantia chlorantha* extracts could potentially serve as an alternative or complement to chloroquine, especially in the context of emerging drug resistance (Evbuomwan et al., 2024).

Another key finding of this study is clear dose-dependent relationship seen between the survival index and the dosage of *Enantia chlorantha* extracts. For the EC2 extract, survival indices were observed to increase with higher doses indicating that EC2 has a beneficial effect on survival in infected mice, with higher doses providing a greater survival benefit. Similarly, EEA extract exhibited a significant dose-dependent effect on survival. At 5 mg/kg, the survival index was relatively low at 9.1%. However, at 10 mg/kg, the survival index surged to 81%, and at 20 mg/kg, it reached an impressive 90%. This marked increase in survival rates with increasing doses of EEA highlights the

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extract's strong potential as an antimalarial agent than other extracts, particularly at higher doses (Ojeaburu & Olasehinde, 2024). The results suggest that EEA might be one of the most potent natural compounds tested, offering a significant life-sustaining effect for infected individuals (Abubakar et al., 2020). In contrast, EHEX showed more moderate improvements in survival rates. At 5 mg/kg, the survival index was 36.4%, and at 10 mg/kg, it increased to 45.5%. However, at 20 mg/kg, the survival rate plateaued at 45.5%, indicating that EHEX's effect on survival might not increase as substantially with higher doses compared to EC2 and EEA. Nonetheless, the survival rates for EHEX were still higher than the negative control (untreated), suggesting a potential therapeutic effect.

These results show that all three extracts from *Enantia chlorantha* (EC2, EEA, and EHEX) have some level of antimalarial activity, as evidenced by increased survival in infected mice. However, the varying degrees of efficacy highlight the differences in potency between the extracts, with EEA emerging as the most effective in terms of survival enhancement. While parasitaemia suppression and chemosuppression are important, survival rates are the ultimate measure of an antimalarial agent's therapeutic efficacy (Apeh et al., 2024). Despite these differences, none of the extracts matched chloroquine in terms of 100% survival outcomes, suggesting that while *Enantia chlorantha* extracts have strong chemosuppressive properties, their effects on overall survival could be improved with better therapeutic approach. Although the extracts did not dramatically change the timing of death in infected mice, the improvement in survival indices observed with increasing doses of EC2, EEA, and EHEX suggests that these treatments could prolong life expectancy in malaria-infected individuals. Hence, this is an important observation, as any therapy that contributes to delayed mortality in malaria cases is a promising step forward, especially in settings where access to conventional treatments might be limited or compromised (Alghamdi et al., 2024). Interestingly, a comparative analysis reveals that while all three *Enantia chlorantha* extracts (EC2, EEA, and EHEX) demonstrated antimalarial effects, their levels of efficacy varied. EEA and EHEX at 20 mg/kg outperformed chloroquine in terms of chemosuppression, suggesting that while chloroquine was more effective in ensuring survival, the *Enantia chlorantha* extracts, particularly EEA, have the potential to reduce parasitaemia more efficiently than the standard treatment in some cases.

Conclusion

In-vivo evaluations with *Plasmodium berghei*-infected mice provided further validation such as the in- silico findings. The significant chemosuppressive activity of these extracts, their ability to reduce parasitaemia levels and to extend survival presumes their potential as transkelotase inhibitors. However, an *in-silico* study could reveal the transkelotase inhibitory role of these extracts.

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