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Phytochemical Profiling and *In vivo* Antimalarial Evaluation of the Stem Bark of *Enantia chlorantha* in *Plasmodium berghei* infected mice

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ABSTRACT

Background*:* Malaria is one of the significant public health diseases in Sub-Saharan Africa with a high mortality rate, especially among underage children and pregnant women. It is transmitted by an infected parasite of the genus Plasmodium, notably *Plasmodium falciparum*. The rate of development of resistance to the current drugs in clinical use to treat the disease is alarming, especially strains resistant to ACTs. There is a need to explore medicinal plants further to find new molecules with potent antimalarial activity, lesser side effects, and effectiveness against resistant strains of *Plasmodium falciparum*. This study aimed to investigate the *in vivo* potential of *Enantia chlorantha,* a medicinal plant used to treat malaria in Nigeria and other African countries.

Methods*: Plasmodium berghei* (NK65) infected mice were treated with methanol extract and fractions of *Enantia chlorantha* using the curative assay method (*Rane's test*). The antioxidant activity of the extract and fractions was determined using the DPPH and FRAP assays and the total phenolic and flavonoid contents.

Results*:* The extract showed the presence of alkaloids, phenolic compounds, flavonoids, saponins, terpenoids, etc., but no tannins and anthraquinones. The total phenolic and flavonoid contents were 86.2±22.42 and 146±5.1 for the extract. The extract showed percentage inhibition in the antioxidant assay with an IC_{50} value of 43 µg/mL compared to the standard ascorbic acid (IC_{50}=0.1 µg/mL). *In vivo,* antimalarial screening revealed that on day 7 post-inoculation, the untreated mice had a 27.6 % percentage parasitaemia, artesunate (0.6%), and ACT showed complete parasitaemia clearance.

Conclusion*:* The findings of this study revealed the extract of *Enantia chlorantha* exhibited a suppressive parasitaemia effect in *Plasmodium berghei*-infected mice after 14 days of treatment post-inoculation, suggesting its potential use in suppressive malaria treatment only.

Keywords: *Enantia chlorantha,* Antioxidants, *Plasmodium Berghei,* Antimalarial, ACTs

1. INTRODUCTION

Malaria, often referred to as a tropical or sub-tropical disease, affects millions of people worldwide, with a case incidence of 263 million globally in 2023, with 597,000 deaths, especially among children under five years of age and pregnant women [1]. It is the primary cause of death in children in Sub-Saharan Africa. Malaria is a parasitic disease caused by plasmodium species and transmitted through a bite from infected female Anopheles mosquitoes. Several species of plasmodium parasite cause malaria in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and the simian *Plasmodium knowlesi* [2]. The most lethal species is *P. falciparum,* which is found predominantly in Africa. If left untreated, *P. falciparum* causes organ failure (severe malaria) and accumulates in the brain capillaries (cerebral malaria), leading to coma and eventually death [3]. Several medicinal plants have been used locally to treat various diseases, including malaria. These plants are known to express potent bioactive compounds, and many medicines in modern medical practice have been developed from these plant sources: *Artemisia annua* L., *Catharanthus roseus* L. (G. Don), and *Taxus brevifolia* Nutt. (Pacific yew), yielding notable antimalarial and anticancer agents, respectively [4,5]. Despite these breakthrough agents, many natural plants are unexplored for new chemical entities that can be used in extract form or synthesized to give potent agents to be used as antimalarial agents. *Enantia chlorantha,* Synonym: *Anickia chlorantha* and *Anickia affinis*, with common names (African yellow wood, African white wood), Local names

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(Awopa, Oso pupa, Dokita-igbo (Yoruba); Osomolu (Ikale), Kakerim (Boki), Erumeru (Nigeria), Erenba-vbogo (Benin), and Moambi jaune (French), [6,7]. In Nigeria, the bark infusion of *E. chlorantha* is employed to treat cough, wounds, typhoid fever, infective hepatitis, jaundice, and rickettsial fever [8]. Other researchers [9] also reported that the stem bark can be used alone to treat malaria as a decoction or infusion, or in combination with one or more other plant species. In the Southeastern part of Nigeria, the stem bark that is boiled or macerated in alcohol treats malaria and may be combined with other plant parts [10]. *E. chlorantha* contains numerous bioactive compounds that justify its numerous medicinal attributes. Phytochemical investigations have reported the presence of bioactive compounds, mostly alkaloids, palmatine, jatrorrhizine, columbamine, and pseudocolumbamine), tetradihydroprotoberberine, 7,8-dihydro-8-hydroxypalmatine, atherosperminine, 7-hydroxydehydronuciferine and 7-hydroxydehydronornuciferine [11-15]. This current study aimed to investigate the *in vivo* antimalarial potential of the extract and solvent fractions of *E. chlorantha* in *Plasmodium berghei*-infected mice, using artesunate and artemether/lumefantrine as the positive control agents or in combination with the plant extract.

2. MATERIALS AND METHODS

2.1 **Materials**

2.1.1 Biological materials

Enantia chlorantha (Annonaceae) plant extract, Healthy Swiss mice bred and maintained at the Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria,

2.1.2 Chemicals and reagents

Acetic anhydride (Sigma-Aldrich, Germany), Alpha-naphthol (Sigma-Aldrich, Germany), Alkaloidal reagents (Drangendorff, Mayer, picric acid and Wagner's reagent) Aluminium chloride hydrate (BDH, England), Ammonium hydroxide (Sigma-Aldrich, Germany), Butanol and Chloroform (JHD, China), 10% Folin ciocalteu's reagent (Sigma-Aldrich, Germany), Distilled water, DPPH (Sigma-Aldrich, Germany), Ethylacetate and Ferric chloride (Qualikems, India), Ferric chloride hexahydrate (Qualikems, India), and TPTZ (2, 4, 6-tripyridyl-s-triazine)(Sigma-Aldrich, Germany). All reagents were of analytical grade. Other materials include Frosted glass slides, Leishman stain, Syringes (1mL, 2mL), and crucibles. Drugs: Artesenute tablets (Greenlife Pharma, Nigeria), Artemether/Lumefantrine combination therapy (ACT).

2.1.3. Apparatus

Rotary evaporator, Water bath, UV spectrometer, Light microscope, Micropipettes

2. METHODS

2.2.1 Collection and preparation of plant materials

Fresh stem barks of *Enantia chlorantha* were purchased from Lagos street market in Benin City, Edo State, Nigeria, in November 2017 and identified at FRIN, Ibadan, where a voucher specimen number FHI102003 was assigned. The plant stem bark was cut into pieces with a knife, dried under shade for 2 weeks, and ground to a powder using an electric blender.

2.2.2 Extraction of plant material

The ground air-dried stem bark (750 g) was extracted with 2.9 L methanol for 48 hours. The extract was concentrated *in vacuo* in a rotary evaporator at 40°C to yield 68.21 g of a dark solid. The extract (60 g) obtained was suspended in 60 mL of water and successively extracted with 500 mL ethyl acetate (EF) and (300 mL) butanol (BF). The mother liquor was unused. The fractions were concentrated and dried under vacuum to yield 6.12 g and 3.12 g for EF and BF, respectively.

2.2.3 Organoleptic Evaluation

The organoleptic property (taste, colour, texture, and odour) of the dried powdered sample of the stem bark of *Enantia chlorantha* was evaluated.

2.2.4 Phytochemical screening of plant sample

Simple chemical tests were conducted on the extract powdered plant material according to standard procedures to identify the phytochemical constituents [16-17]. Approximately 5 g of the extract powdered sample was boiled with 75 mL of distilled water for 30 minutes. The solution was filtered hot and allowed to cool. The filtrate obtained was used to conduct tests for alkaloids, saponins, phytosterols, phenolic compounds, reducing sugars, flavonoids, proteins, and terpenoids.

2.2.5 Determination of Antioxidant Activity

2.2.5.1 Total phenolic content determination (TPC)

The Total phenolic content of the extracts was determined using a previously described method [18]. Briefly, the extract solution (0.5 mL) with a concentration of 1000 µg/mL was added to 4.5 mL of deionized distilled water, followed by 0.5 mL of Folin ciocalteu's reagent (diluted to 1:10 v/v) solution. After mixing, the solution was maintained at room temperature for 5 minutes, followed by adding 5 mL of 7% sodium carbonate and 2 mL of deionized distilled water and incubated for 90 minutes at room temperature. The absorbance was measured spectrophotometrically at 750nm. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). The standard curve was prepared using different concentrations of gallic acid (12.5-150 mg/L).

2.5.2 Total Flavonoid Content Determination

The total flavonoid content of plant extract was determined according to the method previously reported [19]. In this assay, 0.5 mL of extract solution (1 mg/mL) was mixed with 1.5 mL of methanol, and 0.5 mL of aluminium chloride was added, followed by 0.1 mL of 1M potassium and 2.8 mL of distilled water. The mixture was incubated at room temperature for 3 minutes, and the absorbance was measured spectrophotometrically at 415 nm. A standard curve was prepared using quercetin (12.5 – 150 µg/mL). The result was expressed as milligram Quercetin equivalent per gram of extract (mg QE/g extract).

2.2.5.3 DPPH Radical Scavenging Assay

The scavenging effect of the extract and fractions of *Enantia chlorantha* on DPPH radical was estimated using the method previously described [20]. In this method, a solution of 0.2 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of the compounds in methanol containing 0.001 - 0.2 mg/mL of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as the reference standard. The ability of the test samples to scavenge DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[\frac{Ao - A1}{A1}\right] \boldsymbol{x\ 100}$$

Where; A_0 was the absorbance of DPPH radical + methanol,
A_1 = absorbance of DPPH radical + sample extract /standard [21]

The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample required to scavenge 50% of the DPPH free radical, computed from the log-dose response curve [22].

2.2.5.4 Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP stock Solutions (300 mM acetate buffer) were prepared by mixing 3.1 g of sodium acetate trihydrate and 16 mL of glacial acetic acid of pH 3.6, 1 0 mM TPTZ (2,4,6 tripyridyl-s-triazine) solution in 40mM HCl and 20mM $FeCl_3.6H_2O$ solution. The solution was mixed in the ratio 10:1:1 (acetate buffer: TPTZ: $FeCl_3.6H_2O$) and was warmed at 37 ℃ before use. 1.5 mL of extract (0.1 mg/mL) was reacted with 2.8 mL of FRAP solution for 30 minutes under dark conditions. The colour reaction was measured at 593 nm. The standard curve was prepared with $FeSO_4.7H_2O$ in six concentrations (0.01 - 0.015 mM). The result was expressed as millimolar ferrous sulphate equivalent per gram of extract (mM FSE/g Extract).

2.2.6 In Vivo Antimalarial Study

2.2.6.1 Animals

Swiss albino mice (75 male) weighing 18-27 g obtained from Pharmacology Animal House, Faculty of Pharmacy, University of Benin, were used for this study. The mice were maintained under standard laboratory conditions (Temperature: 25-30 ℃, 12-hour light /darkness) and fed with mice pellets (Livestock Feeds, Benin City) and water *ad libitum.* The animal study followed the National Institute of Health Guidelines for Care of Laboratory Animals. The animals were allowed to acclimate for a week before random grouping (Table 1).

2.2.6.2 Acute toxicity (LD50) test of the plant extract

The dose (lethal dose that can cause death to 50% (LD_{50}) of the experimental animals) was determined on the plant extract using Lorke's method [23]. This test was done to observe signs of toxicity in the experimental animals, including clinging together, weakness, anorexia, micturition, respiratory distress, coma, and mortality for the first 24 h and thereafter daily for 14 days.

2.2.6.3 Parasite infection of experimental animals

The chloroquine-sensitive strain of *Plasmodium berghei*-infected (NK65 strain) mice was purchased from the National Institute for Malaria Research, Lagos. The infected mice were kept under standard laboratory conditions (Temperature: 25-30 °C, 12-hour light and 12-hour darkness cycles) with food and water *ad libitum* until the desired level of parasitaemia was achieved. Infected blood from the donor mice was obtained by cardiac puncture after anaesthesia with chloroform and collected into heparinized tubes. The blood was diluted with normal saline so that 0.2 mL contained 1 x 10^7 parasites.

Table 1: Animal groupings and treatment doses

Groups	Doses (mg/kg) b. wt	Groups
1	Control (10% gum Acacia)	Negative control
2	ACT (2/14 mg/kg)	Positive control (artemether/lumefantrine)
3	ART (5 mg/kg)	Positive control (Artesunate)
4	Extract (100 mg/kg)	Extract only
5	Extract (200 mg/kg)	Extract only
6	Extract (400 mg/kg)	Extract only
7	EA (100 mg/kg)	Ethyl acetate fraction
8	EA (200 mg/kg)	Ethyl acetate fraction
9	EA (400 mg/kg)	Ethyl acetate fraction
10	BU (100mg/kg)	Butanol fraction
11	BU (200 mg/kg)	Butanol fraction
12	BU (400 mg/kg)	Butanol fraction
13	CE + ART (400/5 mg/kg)	Extract Extract + Artesunate
14	EA + ART (400/5 mg/kg)	EtOAc fraction + Artesunate
15	BU + ART (400/5 mg/kg)	Butanol fraction + Artesuante

2.2.7.1 Preparation of drugs

Various extract samples were prepared using 10% gum acacia to give a final volume of 15 mL for each fraction and standard drugs (Artesunate and ACT) and their combinations with extract and fractions (Table 1).

2.2.7.2 Curative treatment (Rane's test)

This test was carried out according to an earlier reported method described by Ryley and Peters [24]. Seventy-five mice were infected with 0.2 mL of the diluted infected blood of the donor mice by intraperitoneal injection. Seventy-two hours post-infection (D_0), thin blood smears were prepared from tail blood samples of the animals using Leishman stain. The various groups of mice were appropriately treated via the oral route. The negative control group was treated with acacia gum solution. The drugs were administered to the animals once daily for five days. On the 7th day (D_7), a second thin blood smear was prepared, and the third blood smear was made on the 14th day (D_{14}).

2.2.7.3 Parasite Microscopic Examination

The slides were examined under x100 objective (oil immersion) magnification to determine parasitaemia in 10 random fields. The percentage of parasitaemia was calculated thus:

$$\% \text{ parasitaemia} = \frac{No.\ of\ parasitised\ RBCs}{Total\ No.\ of\ RBCs}\ x\ 100$$

Where RBC = Red blood cells.

2.3 Statistical Analysis

Data are reported as mean ± SD of five treatments. All the statistical analyses were computed with Microsoft Excel statistical software 2025.

3. RESULTS

The organoleptic study was done on the extract powdered drug to evaluate its texture, colour, odour, and taste. The results of this determination show that the sample exhibited a pungent odour and a very bitter taste. Similarly, the results of qualitative phytochemical screening of the aqueous extract of powdered stem bark of *Enantia chlorantha* contained carbohydrates, alkaloids, saponins, tannins, terpenoids, phenolic compounds, and flavonoids, while proteins, steroidal saponins, and anthraquinones are absent (Table 2).

Table 2: Phytochemical constituents of *Enantia chlorantha* stem bark

TEST	Results
Alkaloids	+
Saponins	+
Phenols	+
Carbohydrates	+
Flavonoids	+
Tannins	+
Protein	-
Reducing sugar	+
Diterpenoids	+
Steroidal saponins	-
Anthraquinones	-

NOTE: + present, - absent

Results of the quantitative determination of the content of phenolic and flavonoid compounds in the stem bark extract of EC obtained using the calibration curve equation for gallic acid, Y=0.002X + 0.058, R^2 =0.998 and Quercetin calibration curve, Y = 0.005X + 0.302, R^2= 0.918, respectively and presented in Table 3 and Figure 1.

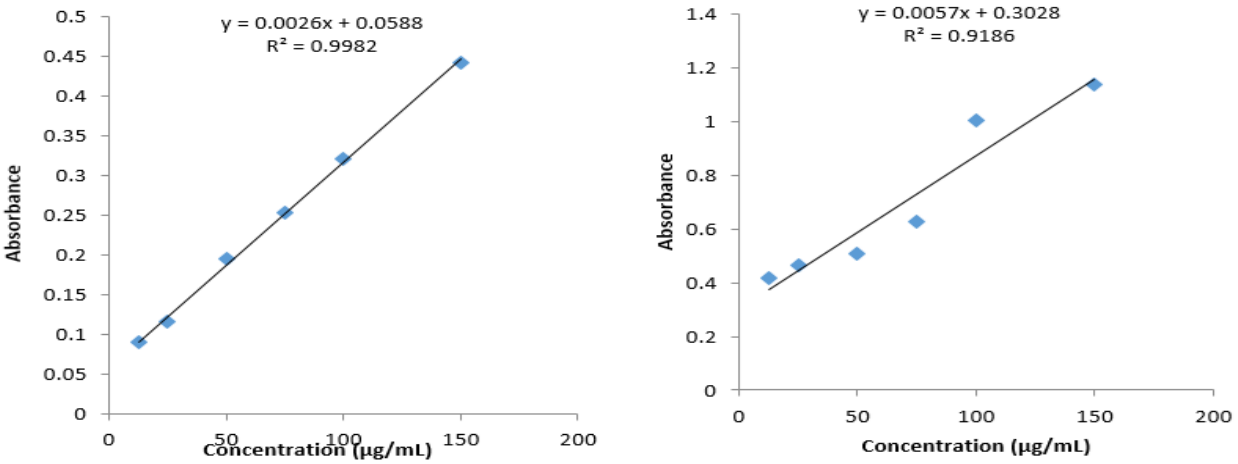
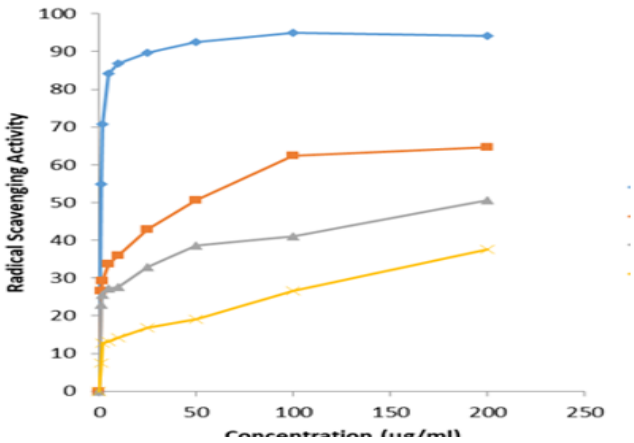


Figure 1: Showing the calibration curves of gallic acid and quercetin for the determination of total phenolic and flavonoid content of the extract and fractions of EC

Table 3: Results of the determination of the Total phenolic and flavonoid contents of extract and fraction of EC stem bark extract.

Fractions	TPC (µg GAE/g of extract)	TFC (µg QE/g of extract)
Extract (Methanol)	86.2±22.42	146±5.4
Ethylacetate	60.8±3.0	147±7.4
Butanol	78.7±1.2	140±5.8

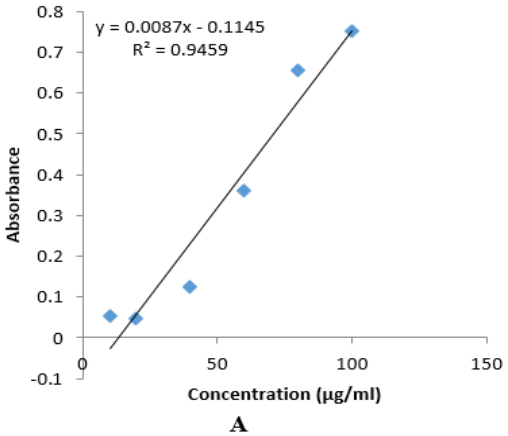
Results of the extracts and fractions antioxidant activities determination using two antioxidant screening models, DPPH (1,1-diphenyl-2-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) assays, are presented in Figure 2. Figure 2a shows the free scavenging activity of the various fractions of *E. chlorantha*, including the standard. Figure 2b shows the 50% inhibitory concentration (IC_{50}) for the fractions and ascorbic acid. In Figure 3a, the ferric chloride-reducing property of EC extract and fractions was obtained from the calibration curve equation of $FeSO_4.7H_2O$ (Y = 0.008x-0.114, R^2 =0.945). The ferric chloride-reducing antioxidant power of the various fractions is shown in Figure 3.



Compounds	IC_{50} values (µg/mL)
Ascorbic acid	0.1
Methanol	43
Ethylacetate	>200
Butanol	>200

Figure 2: (A). Free radical scavenging activity of ascorbic acid, crude extract and fractions of EC, (B) The free radical potential of the standard antioxidant agent (ascorbic acid) and IC50 values of the crude extract and and fractions if EC.

The curative potential of the extract of EC against *Plasmodium berghei* in the *in vivo* antimalarial assay is presented in Figure 4. In all determinations, the extract exerted a dose-dependent effect against the rodent model of plasmodium species.



A

Fractions	FRAP values (µMFSE/g of extract)
Crude (Methanol)	222.08±0.3
Ethylacetate	229±0.3
Butanol	220.58±0.4

B

Figure 3: (A) The calibration curve of FeSO4.7H2O and (B) the ferric chloride reducing antioxant power of the extract and the fractions of EC stem bark.

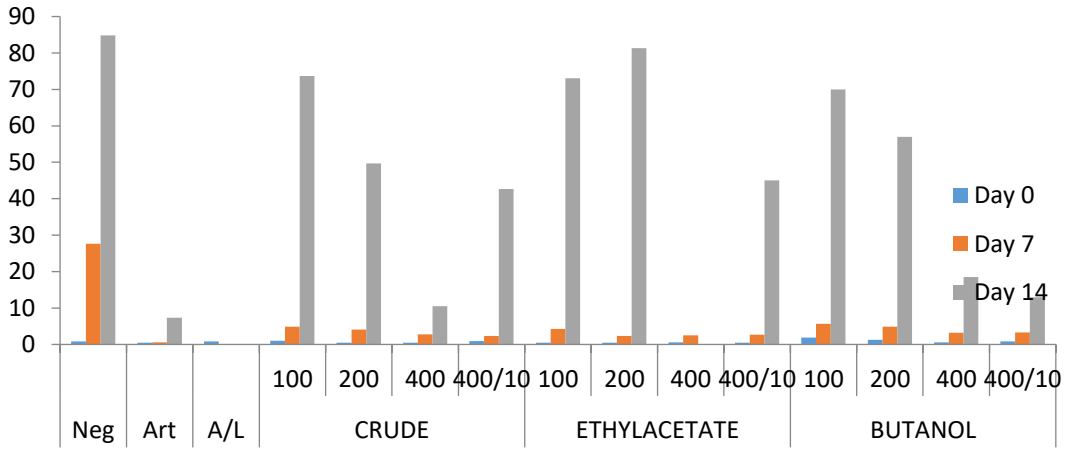


Figure 4: The percentage inhibition of parasitaemia of the extract extract, artesunate (ART), and ACT (artemether/lumefantrine combination) in Plasmodium berghei-infected mice.

Legend: Neg=Negative control (Acacia gum solution), Art=Artesunate and A/L=Artemether-lumefantrine (ACT)

4. DISCUSSION

The evaluation of the organoleptic properties (assessment of sensory characteristics such as colour, taste, odour, and texture) of extracts plays a significant role in determining their quality, especially with herbal materials [25]. It involves the sensory evaluation of the properties of a plant material attributes observed through sight, smell, taste, and touch. In this study, the powdered sample of EC exhibited a very bitter taste, with intense yellow colour, a pungent odour, and a rough, woody texture [26]. Phytochemical analysis of the stem bark of *E. chlorantha* revealed the presence of alkaloids, saponins, phenols, flavonoids, carbohydrates, reducing sugars, and terpenoids (Table 2). Although anthraquinone is reported in a previous study [27], this current did not identify anthraquinone in the setback extract of EC. The alkaloids, phenols, flavonoids, saponins, and terpenoids are called secondary plant metabolites and are responsible for the use of *E. chlorantha* in ethnomedicine for the prophylaxis and treatment of various ailments such as malaria, jaundice, fever, ulcer, wounds, and inflammation [28]. These phytochemicals in EC have been implicated as being responsible for its varied bioactivity and especially its use in the treatment of malaria in Nigeria and other West African countries such as Cameroon, Ghana, Ivory Coast, etc. [29]. The total phenolic content (TPC) of the extract (CF), ethyl acetate (EF), and butanol (BF) fractions of the stem bark of *E. chlorantha* was determined in terms of Gallic Acid Equivalents per g of extract (mg GAE/g), and the results are displayed in Table 3. The CE (extract extract) showed the highest TPC, followed by the BF, with the EF showing the lowest TPC. Phenolics (including many flavonoids) compounds contain polar phenolic hydroxyl groups [30] and the TPC values of the various extracts of *E. chlorantha* increased with increasing polarity: EF (60.8) < BF (78.7) CE (86.2). Under basic reaction conditions, a phenol loses an H^+ to produce a phenolate ion, which reduces the Folin-Ciocalteu reagent [31,32]. The total flavonoid content (TFC) was determined in terms of milligrams of Quercetin Equivalent per g of extract (mg QE/g). The result showed that EF had a slightly higher TFC than CE, while the BF had the lowest TFC (Table 3). All polyphenols do not exist in their free form, and phenolic acids such as ferulic acid and lignans in grains are often bound to structural materials. Hydrolysis using acid or alkali releases these phenolics partitioned into ethyl acetate or n-butanol [18, 33, 34] However, the TPC values obtained in this study are lower than those reported by [35] while the TFC values are comparable. The antioxidant potential of the extracts was determined using the DPPH radical scavenging assay and FRAP methods. DPPH is a rapid, simple, inexpensive, and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of foods [36]. The antioxidant effect is proportional to the disappearance of DPPH in test samples. The DPPH radical inhibition trend by the extracts and the standard ascorbic acid was concentration-dependent, as shown in Figure 2 (a and b). The IC_{50} was calculated from the logarithmic equation of the various fractions and ascorbic acid. The IC_{50} value is used as a correlation of antioxidant activity. IC_{50} is the effective concentration that can scavenge 50% of the DPPH free radical. The lower the IC_{50} value to the standard (ascorbic acid), the better its antioxidant potential. The IC_{50} value of the extracts is ranked in the order CE (43 µg/mL) >EF (>200 µg/mL) > BF (>200 µg/mL). The observed IC_{50} values of the EF and BF were significantly higher than ascorbic acid (0.1 µg/mL),

indicating little or no antioxidant activity, while the extract showed moderate antioxidant activity. FRAP is an inexpensive method to compare antioxidant assays of plant and animal samples. From the experiment, a standard curve was plotted using $FeSO_4.7H_2O$ in increasing concentration. There was an increase in absorbance at 593 nm as concentration increased. The equation derived from the plot of concentration against absorbance was used to calculate the Ferrous sulphate equivalent of the fractions. The higher the FRAP value, the greater the antioxidant potential. The findings from the FRAP assay showed EF (229 µM/g of extract) had the highest value, with BF (220.6 µM/g of extract) showing the lowest value. Various *in vitro* antimalarial studies on *E. chlorantha* have reported antimalarial activity [15,37], which explains why the plant is widely used in Nigerian ethnomedicine for treating malaria. However, there are conflicting reports on the plant's activity in malaria parasite *in vivo* studies. Percentage parasitaemia was used to assess the therapeutic efficacy of the extracts against *P. berghei*-infected mice. The result showed an increase in percentage parasitaemia on day 7 post-treatment across the various groups with a range of 2.3-5.7% compared to the negative control with a percentage parasitaemia level of 27.6%. The positive controls showed a percentage of parasitaemia levels of 0.6% for Artesunate and complete clearance with artemether-lumefantrine. The findings from this study show that the various fractions of the plant suppress the parasite's growth rather than clearing the parasites, as shown in Figure 4. The parasite growth suppression was dose-dependent. On day 14 post-treatment, the percentage of parasitaemia was observed to increase even further in all the treatment groups, including the artesunate group. At the same time, artemether-lumefantrine still maintained complete clearance of parasitaemia. This finding aligns with previous reports [10, 38] where it was stated that the extract was unable to eliminate parasitaemia, was inconsistent with the findings of [39, 26], which reported 100% parasite clearance at a 100 mg/kg dose. However, in both studies, the extract failed to prevent mortality. Adebajo *et al.* [40] reported 49.7% curative activity of *E. chlorantha* on *P. berghei* infected mice compared to chloroquine with 99.3% curative action, while the combination of *E. chlorantha* with *Nauclea latifolia* gave 79.5% curative action. As shown in Figure 4, the combination groups with Artesunate showed a slight decrease in the suppressive capacity of Artesunate, suggesting a potential negative interaction. Same researchers also reported a negative interaction of the extract with chloroquine, where chloroquine alone gave 99.3% curative effect and 81.2% activity in combination with *E. chlorantha* [40]. The inability of the plant to clear malaria parasites could be the reason for the combination of the plant with other plants in Nigeria's ethnomedicine [41,10].

5. CONCLUSION

This study has revealed that the stem bark of *E. chlorantha* contains alkaloids, phenols, flavonoids, saponins, tannins, terpenoids, carbohydrates, and reducing sugars. The methanol extract also exhibited moderate antioxidant potential. The antimalarial assay showed good parasite suppression, but the extracts could not eliminate the parasites. The combination of the extract with Artesunate resulted in decreased suppressive ability. The study highlighted that artemether/lumefantrine combination therapy still has the potential for better antimalarial efficacy. The study recommends that *E. chlorantha* should not be used with the artemisinin derivatives or for treating severe malaria due to observed drug-herb interactions with possible treatment failure.

Declarations

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Conflict of Interest: The authors have declared that no conflict of interest exists.

Contribution of the Authors: VOI = designed, conceptualized, and supervised the work and drafted and proofread the original manuscript. CBO = performed the experiments, drafted the manuscript, and analyzed data.

6. REFERENCES

[1] WHO (2024), https://www.who.int/data/gho/data/themes/malaria

[2] Snow RW. Global malaria eradication and the importance of Plasmodium falciparum epidemiology in Africa. *BMC Med.* 2015;13:23. doi: 10.1186/s12916-014-0254-7.

[3] Battle KE, Gething PW, Elyazar IR, Moyes CL, Sinka ME, Howes RE, Guerra CA, Price RN, Baird JK, Hay SI. The global public health significance of *Plasmodium vivax. Adv. Parasitol*. 2012;80:1-11.

[4] Davis CC, Choisy P. Medicinal plants meet modern biodiversity science. Curr. Biol. 2024;34(4):R158-73.

[5] Pirintsos S, Panagiotopoulos A, Bariotakis M, Daskalakis V, Lionis C, Sourvinos G, Karakasiliotis I, Kampa M, Castanas E. From traditional ethnopharmacology to modern natural drug discovery: A methodology discussion and specific examples. *Molecules.* 2022;27(13):4060.

[6] Akinwale SG, Chukwu OE, Chioma OP, Chukudi AJ, Olubunmi AG. *Enantia chlorantha*: A review. *J. Pharmacogn. Phytochem.* 2022;11:34-8.

[7] Olivier D, Van Vuuren S, Moteetee A. *Annickia affinis* and *A. chlorantha* (*Enantia chlorantha*) – a review of two closely related medicinal plants from tropical Africa. *J. Ethnopharmacol.* 2015; 176:438-462.

[8] Gill IS and Akinwunmi C. Nigerian Folk Medicine: Practices and Beliefs of the Ondo People. *J. Ethnopharma.* 1986. 18(3): 259 – 266.
[9] Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO. Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria, African. Afr. J. Tradit. Complement. Altern. Med. 2007; 4(2):191-198.

[10] Ogbonna DN, Sokari TG, Agomuoh AA. Antimalarial activities of some selected traditional herbs from South Eastern Nigeria against Plasmodium species. *J. Parasitol. Res*. 2008; 3(1): 25–31.
[11] Hamonnière M, Leboeuf A, Paris RR. Alcaloïdes des annonacées: Alcaloïdes de *Enantia chlorantha* Olivier. *Planta Medica*. 1975; 9(4), 296–303

[12] Jalander L, Sjöholm R, Virtanen P. Protoberberine alkaloids from the bark of *Enantia chlorantha.* Collect. Czechoslov. *Chem. Commun*. 1990; 55: 2095–1178.
[13] Tan PV, Nyasse B, Enow-Orock GE, Wafo P, Forcha EA. Prophylactic and healing properties of a new anti-ulcer compound from *Enantia chlorantha* in rats. *Phytomedicine.* 2000 ;7(4):291-6.
[14] Wafo P, Nyasse B, Fontaine C. A 7,8-dihydro-8-hydroxypalmatine from *Enantia chlorantha*. *Phytochemistry*. 1999a; 50: 279–281.

[15] Imieje V, Zaki AA, Fasinu PS, Ali Z, Khan IA, Tekwani B, Khan SI, Nosa EO, Falodun A. Antiprotozoal and cytotoxicity studies of fractions and compounds from *Enantia chlorantha*. *TJNPR*. 2017;1(2):89-94.
[16] Ayeni MJ, Oyeyemi SD, Kayode J, Peter GP. Phytochemical, proximate and mineral analyses of the leaves of *Gossypium hirsutum* L. and *Momordica charantia* L. *J. Nat. Sci. Res*. 2015;5(6):99-107.
[17] African Pharmacopoeia, 2014. http://archives.au.int/handle/123456789/1845
[18] Kim KH, Tsao R, Yang R, Cui SW. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food. Chem.* 2006; 95: 466-473

[19] Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol, and flavonoid content of some medicinal plants from Iran. *Afr. J. Biotechnol.* 2008;7(18).

[20] Jain A, Soni M, Deb L, Jain A, Rout SP, Gupta VB, Krishna KL Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Mormordica dioica* Roxb leaves. *J. Ethnopharmacol*. 2008; 115 (1): 61-66.
[21] Fogliano V, Verde V, Randazzo G, Ritieni A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J Agric Food Chem*. 1999; 47: 1035-1040.
[22] Roach JS, McLean S, Reynolds WF, Tinto WF. Cassane diterpenoids of *Caesalpinia pulcherrima*. *J. Nat. Prod.* 2003; (66): 1378-1383.
[23] Lorke D. A new approach to practical acute toxicity testing. *Arch. Toxicol*. 1983 ;54:275-87.

[24] Oladeji OS, Oluyori AP, Dada AO. Antiplasmodial activity of *Morinda lucida* Benth. Leaf and bark extracts against *Plasmodium berghei*-infected mice. *Saudi J Biol Sci.* 2022;29(4):2475-2482. doi: 10.1016/j.sjbs.2021.12.017.

[25] Kawale KA, Autade NB, Narhare SH, Mhetrea RL. A review on fast-dissolving oral film. *Asian J Pharm Clin Res*. 2023;7:17.

[26] Adesokan AA, Akanji MA. Antimalarial bioactivity of *Enantia chlorantha* stem bark. *Pharmacol. Ther.* 2010: 441-447.

[27] Bassey AI, Ettebong EO. Anti-oxidant, Histopathology and LD50 Evaluation of Ethanol Stem Bark Extract of Enantia chlorantha in Rodents. *Med Aromat Plants*. 2017;6(300):2167-0412.

[28] Kuissu Mimosette M, Enow-Orock George E, Mezui Christophe N, Ernestine Z, Benjamin N, Siwe Gael T. Evaluation of the toxicity of the aqueous stem bark extract of *Enantia chlorantha* on some reproductive and developmental parameters. *J. Med. Plants Stud.* 2020; 8(2): 86-94

[29] Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO. Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria, *Afr. J. Tradit. Complement. Altern. Med.* 2007; 4(2):191-198.

[30] Dildar, A., Muhammad, M.K., Ramsha, S. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexane and aqueous extracts from *Adiantum caudatum* leaves. US National Library of Medicine, *National Institute of Health.* 2015; 4(2): 394-409.

[31] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *J. Agric. Food. Chem.* 2005; 53:1841-1856.

[32] Fernandes, A.J.D., Ferreira, M.R.A., Randau, K.P., Souza, T.P., Soares, L.A.L. Total flavonoid content in the raw material and aqueous extractives from *Bauhinia monandra* Kurz (Caesalpiniaceae). *Sci.World J. 2012*, 99234622, doi: 10.1100/2012/932462.

[33] Adom KK, Liu RH. Antioxidant activity of grains. *J. Agric. Food Chem*. 2002; 50, 6182-6187.

[34] Chandrasekara, A., Shahidi, F. Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *J. Agric. Food. Chem*. 2010; 58, 6706-6714.

[35] Olanlokun, J.O., Akomolafe, S.F. Antioxidant potentials of various solvent extracts from stem bark of *Enantia chlorantha*. *JBiSE.* 2013; 6: 877–884.

[36] Prakash D, Upadhyay G, Pushpangadan P, Gupta C. Antioxidant and free radical scavenging activities of some fruits. *J. Complement. Integr. Med*. 2011;8(1):1-6.

[37] Vennerstrom, J.L., Klayman, D.L. Protoberberine alkaloids as antimalarials. *J. Med. Chem*. 1988; 31: 1084–1087.

[38] Agbaje EO, Onabanjo AO. The effects of extracts of *Enantia chlorantha* in malaria. *Ann. Trop. Med. Parasitol.* 1991; 85(6), 585–590.

[39] Kimbi HK, Fagbenro-Beyioku AF. Efficacy of *Cymbogon giganteus* and *Enantia chlorantha* against chloroqiune resistant *Plasmodium yoeliinigeriensis*. *East Afr. Med. J.* 1996; 73(100): 636–637.

[40] Adebajo AC, Odediran SA, Aliyu FA, Nwafor PA, Nwoko NT, Umana US. *In vivo* antiplasmodial potentials of the combinations of four Nigerian antimalarial plants. *Molecules*. 2014;19(9):13136-46.

[41] Adeyemi AA, Gbolade AA, Moody JO, Ogbole OO, Fasanya MT. Traditional anti-fever phytotherapies in Sagamu and Remo north districts in Ogun State, Nigeria. *J. Herbs Spices Med. Plants*. 2010;16(3-4):203-18.