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Antimalarial activities of *Alchornea laxiflora* stembark extract and fractions in *Plasmodium berghei*-infected mice

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Abstract

Alchornea laxiflora [Benth.] Pax and K.Hoffm(Euphorbiaceae) is used in Ibibio ethnomedicine for the treatment of various diseases such as malaria. The stembark extract and fractions of *Alchornea laxiflora* (141 - 424 mg/kg) were investigated for antimalarial activity against *Plasmodium berghei* infection in mice using standard experimental models; suppressive, prophylactic and curative tests. Acute toxicity study of the extract and phytochemical analysis were carried out. The stembark extract (141 - 424 mg/kg, p.o.) with LD_{50} of 1414.21mg/kg exerted significant ($p<0.05–0.001$) antimalarial activity against *P. berghei* infection in suppressive, prophylactive and curative tests with methanol and dichloromethane fractions having the highest activity. Phytochemical screening revealed the presence of active compounds. These results suggest that the stembark extract/fractions of *Alchornea laxiflora* possess antimalarial activities which justify its use in ethnomedicine to treat malaria.

Keywords: Malaria; *Alchornea laxiflora; Plasmodium berghei*; herbal medicine

1. Introduction

Malaria has continued to threaten lives of millions of people globally with Africa bearing the major burden of the disease. World malaria report of 2023 showed that Nigeria is the most affected African country with 25.9% of the global malaria cases in 2023 and 30.9% of all global malaria deaths especially in children occurring in Nigeria alone [1]. Poverty and poor economy have been advanced to be responsible for the high burden of malaria in Africa coupled with the high cost of active artemisinin combination therapies (ACTs) and inaccessibility to health facilities. Health challenges due to malaria is further complicated with the emergence of partial resistance to artemisinin in some parts of Africa [1], indicating serious threat to lives in Africa and Nigeria in particular. Hence, the search for active, safe and cost friendly remedy to malaria which can be found in natural products such as medicinal plants.

Alchornea laxiflora [Benth.] Pax and K.Hoffm (Euphorbiaceae) is a perennial small tree that grows in most parts of Africa including Nigeria, Congo, Ethiopia, and throughout East Africa to Zimbabwe [2]. *A. laxiflora* is called “Opoto” and “Nwariwa,” respectively, among the Yoruba and Ibibio tribes of Nigeria. Stem bark and branches have also been used in traditional medicine for various purposes, notably for malaria, anemia, emmenagogue, ringworm, venereal disease, typhoid fever, antioxidant, infertility in females, infectious diseases, tumor, inflammation, teething problems, and toothache in South Africa, Ghana, and Nigeria [3].

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Biological activities reported of the stembark include antioxidant [4,5], anti-HIV, antibacterial and cytotoxic activities [6], larvicidal effect [7], anticholinesterase activity [8], antibacterial activity against multi-drug resistant (MDR) bacteria, including strains of *E. coli* (ATCC 8739, AG102, AG100 Atet), *Enterobacter aerogenes (E. aerogenes)* (ATCC 13048, CM64, EA27), *K. pneumoniae* (ATCC 11296, KP55), *Providencia stuartii* (*P. stuartii*) (ATCC29916, PS299645), *Enterobacter cloacae* (*E. cloacae*) (BM47, BM67), and *P. aeruginosa* [9] and analgesic activity [10]. The stem bark of *Alchornea laxiflora* contains various phytochemicals, including fatty acid derivatives, ellagic acid and its derivatives, and triterpenoids. The phytochemical investigation of methanolic extract from the stem bark of *A. laxiflora* resulted in the isolation of some compounds, including ellagic acid, 3-O-methylellagic acid, and 3-O-methylellagic acid-3-O-α-rhamnopyranoside [11]. A novel ellagic acid derivative, namely, 3,4,3′-tri-O-methylellagic acid, was isolated from the methanolic extract obtained from the *A. laxiflora* stem bark [9]. Sandjo *et al.* [11] and Tapondjou *et al*. [12] isolated and established the structure of a known steroidal glycoside, β-sitosterol-3-O-β-D-glucopyranoside from the methanol extract of stem bark, ellagic acid; 3-O-methyl-ellagic acid , 3-O-β-D-glucopyranosyl-β-sitosterol , 3-O-acetyloleanolic acid and 3-O-acetyl-ursolic acid. Several pentacyclic triterpenoids, such as 3-acetyloleanolic acid, 3-acetoxyursolic acid, adipedatol, and betulin, have been found in the stem bark. Squalene and 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl heptadeca-3,7,11,15-tetraenyl) -cyclohexanol have also been identified as triterpenoids in the stembark extract [11]. This study investigated the antimalarial activity of the ethanol stembark extract of *A. laxiflora.*

2. Materials and Methods

2.1. Plants collection

The plant material *Alchornea laxiflora* (stembark) were collected in bushes in Uyo area, Akwa Ibom State, Nigeria in April 2024. The plant was identified and authenticated by a taxonomist in Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

2.2. Extraction

The stembarks were washed and shade dried for two weeks. The dried stembarks were cut into smaller pieces and pulverized to powder. The stembark powder was divided into two parts. One part was macerated in ethanol for 72 hours, while the remaining part was successively and gradiently macerated for 72 hours in each of, *n*-hexane, dichloromethane, ethyl acetate and methanol respectively, which is along their polarity to give the corresponding gradient fraction for each solvent. The liquid filtrate of the extract and fractions were concentrated and evaporated to dryness in *vacuo* 40^0C using a rotary evaporator. The various yields were calculated and the extract and fractions were stored in a refrigerator at -4^0C, until used for the proposed experiments.

2.3. Phytochemical Screening

Phytochemical screening of the crude extract was carried out employing standard procedures and tests [13], to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, and cardiac glycosides and others.

2.4. Microorganism (parasite)

Chloroquine-sensitive strain of *Plasmodium berghei* ANKA strain was obtained from the National Institute of Medical Research (NIMR), Yaba Lagos, Nigeria and maintained by subpassage of blood from infected to healthy mouse once every 7-8 days.

2.5. Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of infected blood containing about 1 x 10^7*P. berghei* parasitized erythrocytes collected from an infected mice with 20-30% parasitaemia. The inoculum consisted of 5 x 10^7 *P. berghei* infected erythrocytes per milliliter prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations [14,15]. Parasitemia was monitored by standard methods; thin blood smears were made on glass slides, fixed using methanol, and stained using Giemsa stain. The parasitemia was counted using a microscope and was calculated as a percentage of infected red blood cells (RBCs) relative to the total number of cells in a microscopic field at ×100 magnification according to the formula of Okokon and Nwafor [14] as given below:

$$\text{Parasitemia (\%)} = \frac{\text{Total number of parasitised RBCs}}{\text{Total number of RBCs}} \times 100$$

2.6. Experimental animals

Swiss albino mice (18-25 g), male and female, used in the study were obtained from the University of Uyo's Animal house. They were kept in standard plastic cages in a well ventilated room and left to acclimatized for a period of 10 days before the experiments. The mice were fed on standard pelleted diet and water *ad libitum*. The care and use of animals were conducted in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH Publication, 1996). Approval for the study was obtained from the University of Uyo's Animal Ethics Committee.

2.7. Drug administration

The extract, fractions, chloroquine and pyrimethamine that were used in the study were administered orally with the aid of a stainless metallic feeding cannula.

2.8. Determination of median lethal dose (LD_{50})

The determination of median lethal dose (LD_{50}) of the extract was carried out in mice using oral (p.o) route by modified method of Lorke [16]. The animals in groups of three mice each were administered different doses of the extract (100– 5000 mg/kg). They were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/ limb tone, and decreased mobility and death. The mortality in each group within 24 h was recorded. The LD_{50} value was calculated as geometrical mean of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b). $LD_{50} = \sqrt{ab}$

2.9. Evaluation of the in vivo antimalarial activities of stembark extract and fractions of Alchornea laxiflora:

2.9.1. Evaluation of Suppressive Activity (4-day Test) of stembark extract and fractions of Alchornea laxiflora.

The schizontocidal activity of the crude extract and fractions was evaluated against early *P. berghei berghei* infection in mice. This was conducted according to the methods previously described by Okokon *et al.* [17] and Okokon *et al.* [18]. Fifty four mice were randomly divided into nine groups of six mice each. On the first day (D_0), the Fifty four mice were infected with the parasite and randomly divided into various groups. These were administered the crude extract, fractions and chloroquine. Groups 1 - 3 received 141, 282 and 424 mg/kg/day of the stembark extract, respectively. Groups 4, 5, 6 and 7 were given 282 mg/kg of *n*-hexane, dichloromethane, ethyl acetate, and methanol, respectively. Groups 8 and 9 were respectively administered 5 mg/kg/day of pyrimethamine (positive control) and 10mL/kg of distilled water (negative control).The mice in the various groups were treated with standard drug, extract and fractions for four consecutive days (D_0 – D_3) between 8am and 9am. On the fifth day (D_4), thin blood films were made from the tail blood of the experimental animals. The films were stained with Giemsa stain to reveal parasitized erythocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls(14). The mean survival time of the animals was determined over a period of 30 days.

2.9.2. Evaluation of Prophylactic or Repository Activities of of stembark extract and fractions of Alchornea laxiflora.

The repository activity of the extract and pyrimethamine was assessed using the method described by Okokon *et al.,* [19]. The mice were randomly divided into nine groups of six mice each. Groups 1 - 3 received 141, 282 and 424 mg/kg/day of the stembark extract, respectively. Groups 4, 5, 6 and 7 were given 282 mg/kg of *n*-hexane, dichloromethane, ethyl acetate and methanol, respectively. Groups 8 and 9 were respectively administered 5 mg/kg/day of pyrimethamine (positive control) and 10 mL/kg of distilled water (negative control). Administration of the extract, fractions and drug was done for three consecutive days (D_0 – D_2). On the fourth day (D_3) the mice were inoculated with *P. berghei.* The parasitaemia level was assessed by blood smears seventy-two hours later and average percentage suppression of parasitaemia was calculated in comparison with the control (14). The mean survival time of the animals was determined over a period of 30 days.

2.9.3. Evaluation of Curative Activities of Extract (Rane's test) of stembark extract and fractions of Alchornea laxiflora.

This test was used to evaluate the schizontocidal activity of the extract, fractions and chloroquine in established plasmodial infection. This was conducted according to the methods described by Okokon et al., [19]. *P. berghei berghei* was injected intraperitoneally into fifty-four (54) mice on the first day *(D_0)*. Seventy two hours later (D_3), the mice were divided into nine groups of six mice per group. Groups 1-3 were given different doses of extract, 141, 282 and 424 mg/kg, respectively, groups 4-7 were given 282 mg/kg of n-hexane, dichloromethane, ethyl acetate and methanol fractions respectively, group 8 was given 5 mg/kg/day chloroquine (positive control) and group 9 was given 10 mL/kg distilled water (negative control). The crude extract, fractions and chloroquine were administered once daily for 5 days. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor the parasitemia level. The average percentage suppression of parasitaemia was calculated in comparison with the control.

The mean survival time (MST) of the mice in each group was determined over a period of 29 days (D_0-D_{28}). Rectal temperatures of the mice were taken on days 0, 3, 5, and 7.

2.10. Statistical analysis

Data collected were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean ± SEM and significance relative to control were considered at $p<0.001$ and $p<0.05$.

3. Results

3.1. Yields of extract and fractions

The percentage yields of the extract and fractions were; crude-20.01%, *n*-hexane- 0.22%, dichloromethane -0.82%, ethyl acetate -0.32%, methanol -2.1%.

3.2. Phytochemical screeening

The results of the phytochemical screening of the stembark extract of *A. laxiflora* revealed that the extract contains alkaloids, phenol, terpenes, saponins, flavonoids and reducing sugars.

3.3. Determination of Median Lethal Dose (LD_{50})

The median lethal dose determined by administration of different doses of the ethanol stem bark extract of *Alchornea laxiflora* to Swiss albino mice in groups of three mice each was 1414.21mg/kg. The minimum dose that produce 100% mortality was 2000 mg/kg, while the maximum dose that produced 0% mortality was 1000 mg/kg. The LD_{50} was calculated to be 1414.21mg/kg.Physical signs of toxicity observed included restlessness, paw licking, gasping, decreased motor activity and death.

3.4. Suppressive activities of ethanol stembark extract and fractions of *A. laxiflora*

The stembark extract and fractions exerted dose-dependent reductions in parasitaemia of the treated mice in various treatment groups. The reduction was only statistically significant($p<0.01$) at the higher doses of the extract (282 and 424 mg/kg), DCM and methanol fractions treated groups when compared to the control. Methanol fraction exerted the highest suppressive effect (33.33%) and m.s.t value of 18.96 d followed by DCM fraction with chemosuppression of 32.22% and m.s.t of 19.02 d which was not comparable to that of the standard, Chloroquine, 5 mg/kg (77.94 %)(Table 1).

3.5. Prophylactic/repository activities of ethanol stembark extract and fractions of *A. laxiflora*

The stembark extract exerted dose-dependent reductions of parasitaemia in the extract-treated groups. These reductions were statistically significant relative to the control($p<0.01–0.001$) at the highest dose of the stembark extract (424 mg/kg). The methanol fraction showed the most prominent prophylactic activity (36.13%) and m.s.t value of 16.00 d which was significant ($p<0.05$) when compared to control but was not comparable to that exhibited by the standard drug, pyrimethamine, 1.2 mg/kg (Table 2).

3.6. Antiplasmodial effect of ethanol stembark extract and fractions of *A. laxiflora* on established infection

There were dose-dependent reductions of parasitaemia in the extract/fraction-treated groups progressively relative to control. These reductions were statistically significant relative to the control($p<0.001$) especially in the groups treated with the extract's high dose (424 mg/kg), DCM, ethyl acetate and methanol fractions. The methanol fraction had the highest activity with chemosuppressive effect of 65.14 % on day 7, this was lower compared with that of the standard, chloroquine,92.24% (Figure 1). The stembark extract and fractions demonstrated significant (p<0.05-0.001)protective potentials on the mice as was seen in the mean survival time of the animals. The groups treated with methanol fraction had a longer mean survival time, 17.46 ± 1.72 d followed by those of dichloromethane fraction treated mice, 16.54 ± 0.33 d. These were less than that of the standard drug, chloroquine (29.17 ± 0.13 d)(Table 3).

3.7. Effect of stembark Extract/Fractions on Rectal Temperatures of infected mice

Administration of the stembark extract and fractions of *A. laxiflora* as well as chloroquine to *Plasmodium berghei*-infected mice in curative test did not cause any significant difference (p>0.05) in the rectal temperatures of the treated mice when compared with that of control in the this model (Table 4).

Table 1 Suppressive activities of stembark extract and fractions of *A. laxiflora* during early *Plasmodium berghei* infection in mice

Treatment	Dose (mg/kg)	Parasitaemia (%)	Chemosuppression (%)	MST
Control	-	24.33 ±0.15	-	12.45 ±0.83
Extract	141	21.25±1.14	12.65	14.23±0.55
	282	18.10±0.55[a]	25.60	15.13±0.26
	424	16.44±0.62[a]	32.42	18.28±0.56[c]
n-hexane	282	20.81 ±0.34	14.46	13.46±0.72[b]
Dichloromethane	282	16.33±0.61[a]	32.88	19.02±0.66[b]
Ethyl acetate	282	20.67±0.45	15.04	14.74±0.38[c]
Methanol	282	16.22 ± 0.58[c]	33.33	18.96±0.76[b]
Chloroquine	5	2.10 ± 1.38[c]	91.36	30.00 ±0.00[c]

Values are expressed as mean ± SEM. Significant relative to control. [a]p<0.05; [b]p<0.01; [c]p<0.001. n = 6

Table 2 Prophylactic activities of stembark extract and fractions of *A. laxiflora*

Treatment	Dose (mg/kg)	Parasitaemia(%)	Chemosuppression (%)	MST(days)
Control	-	21.42±0.34	-	11.63 ±0.24
Extract	141	19.49±0.35	9.01	11.03±0.44
	282	17.23±1.32	19.56	12.20±0.53
	424	14.96±1.12[a]	30.15	14.38±0.82[a]
n-hexane	282	16.33±0.34	23.76	13.50 ±0.68
Dichloromethane	282	14.02±0.77[b]	34.54	15.84 ±1.87[a]
Ethyl acetate	282	17.36±0.46	18.95	12.78 ±0.54
Methanol	282	13.68±1.76[c]	36.13	16.00 ±1.45[a]
Pyrimethamine	1.2	2.21 ± 0.86[c]	89.68	24.89 ±0.48[c]

Values are expressed as mean ± SEM. Significant relative to control. [a]p<0.05; [b]p<0.01; [c]p<0.001. n = 6.

Table 3 Mean survival time of mice treated with stembark extract and fractions of *A. laxiflora* during established *Plasmodium berghei* infection in mice

Treatment	Dose (mg/kg)	Mean Survival Time (Days)
Control	-	11.30 ±0.18
Extract	141	12.50±0.44
	282	13.88±0.28[a]
	424	16.63±1.35[c]
n-hexane	282	14.55±1.26[a]
Dichloromethane	282	16.54±0.33[c]
Ethyl acetate	282	15.33±1.34[b]
Methanol	282	17.46±1.72[c]
Chloroquine	5	29.17±0.13[c]

Values are expressed as mean ± SEM. Significant relative to control. [a]p<0.05; [b]p<0.01; [c]p<0.001. n = 6.

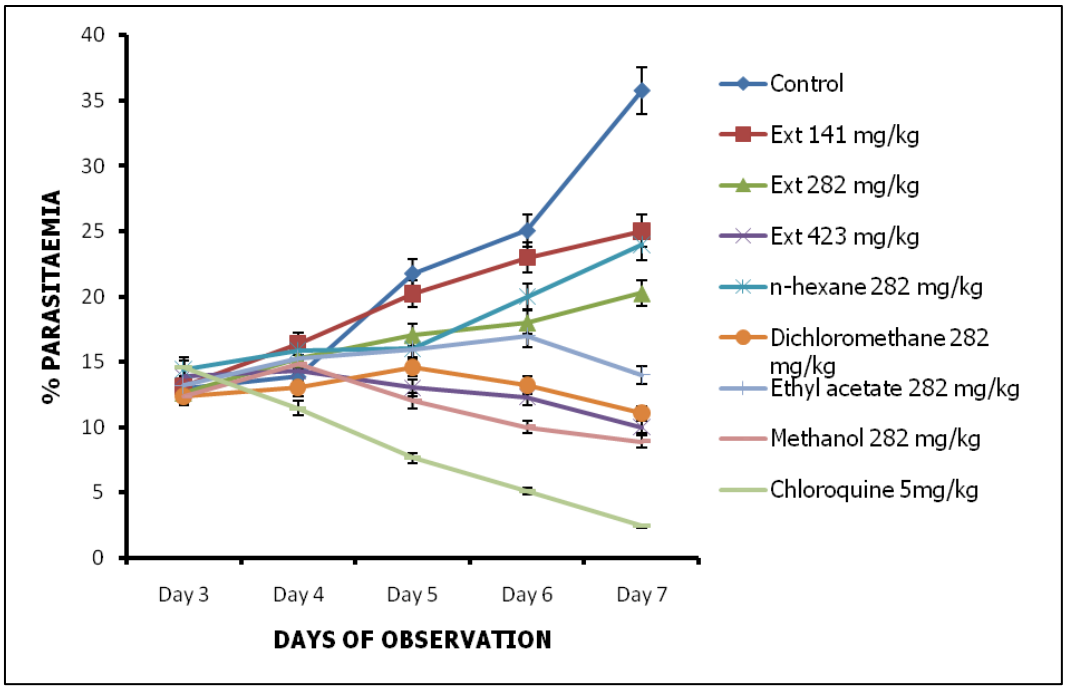

Figure 1 Effect of stembark extract and fractions of *A. laxiflora* on established *Plasmodium berghei* infection in mice

Table 4 Effect of stembark extract and fractions of *A. laxiflora* on rectal temperatures of mice infected with *Plasmodium berghei* during established infection

Treatment	Dose (mg/k)	Rectal Temperature (°C)			
		D0	D3	D5	D7
Control	-	35.86±0.46	36.22 ±0.02	36.22 ±0.02	36.75 ±0.04
Extract	141	36.47±0.07	36.52±0.03	36.53±0.02	36.42±0.05
	282	36.41±0.07	36.43±0.02	36.33±0.02	36.30±0.04
	423	36.50±0.04	36.32±0.02	36.32±0.02	36.23±0.02
n-hexane	282	36.47±0.04	36.40 ±0.02	36.30 ±0.02	36.50 ±0.04
Dichloromethane	282	36.52±0.04	36.30± 0.06	36.40± 0.04	36.33 ±0.06
Ethyl acetate	282	36.52±0.02	36.45± 0.04	36.33± 0.06	36.30 ±0.06
Methanol	282	36.00±0.20	36.30± 0.10	36.30± 0.10	36.31 ±0.04
Chloroquine	5	36.18 ±0.27	36.37 ±0.07	36.37 ±0.07	36.22±0.08

Values are expressed as mean ± SEM.

4. Discussion

The stembark of *Alchornea laxiflora* is used in traditional medicine as remedy for diabetes, fever, inflammatory diseases and pain among others. This investigation was designed to confirm and authenticate the antimalarial potential of the stembark extract and fractions of *A. laxiflora* in order to provide scientific basis for its usage in traditional medicine. The stembark extract and fractions of *A. laxiflora* were investigated for antimalarial activity against rodent malaria parasite, *P. berghei* infection in mice using standard *in vivo* models. It was found that the extract and fractions significantly reduced the parasitaemia in suppressive, prophylactic and curative models in a dose-dependent fashion with methanol fraction exhibiting the strongest *in vivo* suppressive, prophylactic and curative potentials followed by dichloromethane fraction, thus confirming the antimalarial potential of this extract. The extract and fractions also prolonged the MST of the mice, but was unable provide complete protection as most of the treated animals eventually died from infection suggesting that the extract and fractions lacks the potentials to protect the mice completely. This activity could have resulted from the inability of the stembark extract and fractions to kill the parasites or effectively suppress their growth and development consequent of weak plasmodicidal or plasmodistatic activity of the extract and fractions. This results corroborate previous reports on antimalarial activities of the leaf and root extracts of *A. laxiflora* [20, 21]. These results validate the use of the stembark extract decoctions as malarial remedy.

The moderate suppressive activities of the extract/fractions on the development of *Plasmodium berghei* parasitaemia as observed during *in vivo* study, suggests the inability of the extract and fractions to effectively suppress or eliminate the erythrocytic stages of the parasite [22]. The inability of the extract/fractions to exert complete protection to the infected animals in most cases could have resulted from low doses (141 - 424 mg/kg) used, short half life/ duration of action of the extract/fractions due to rapid biotransformation processes and subsequent elimination [22]. Thus, resulting in further development and multiplication of the parasites as well as short MST of the treated mice as observed in this study.

The phytochemical screening of the stembark extract revealed the present of some pharmacologically active compounds such as tannins, flavonoids, alkaloids, terpenes, phenols among others. Reported phytoconstituents of the stembark which include fatty acid derivatives, ellagic acid and its derivatives and triterpenoids such as ellagic acid, 3-O-methylellagicacid, and 3-O-methylellagic acid-3-O-α-rhamnopyranoside [11], 3,4,3′-tri-O-methylellagic acid [9], β-sitosterol-3-O-β-D-glucopyranoside, 3-O-β-D-glucopyranosyl-β-sitosterol, 3-O-acetyloleanolicacid and 3-O-acetyl-ursolic acid, 3-acetyloleanolic acid, 3-acetoxyursolic acid, adipedatol, betulin, squalene and 2,2,4-trimethyl-3-(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)-cyclohexanol [11,12], are likely to be responsible for the observed activities of the extract and fractions. However, secondary metabolites of plants such as alkaloids, flavonoids and triterpenoids have been documented previously to exhibit antiplasmodial properties [23,24]. Polyunsaturated fatty acids found in this stembark extract have been implicated in antiplasmodial activity which has been correlated with increased degree of unsaturation [25,26,27,28.29,30]. Flavonoids present in the stembark have been reported to exert significant antiplasmodial activity against various strains of *P. falciparum* [31,32,33], while squalene, an active

antioxidant compounds [34,35,36] present in the extract may also be responsible for the observed antiplasmodial activities. Flavonoids are reported to exert antiplasmodial activity through free radical scavenging mechanism[32,37], chelation of nucleic acid base pairing of the parasite [38], modulation of host immunity to tackle disease and inhibition of plasmodial enoyl-ACP reductase (FAB I enzyme) – a key regulator of type II fatty synthases (FAS-II) in *P. falciparum* [39,40] as well as binding to parasite's serinethreonine kinase with high affinity thereby affecting its development [41]. The stembark extract/fractions may be acting through one of these mechanisms. These compounds present in this stembark extract and fractions may in part have contributed to the plasmodicidal activity of this extract/fraction. The findings of this study suggest that stembark extract and fractions of *A. laxiflora* possess antimalarial activity which is due to the activities of its phytochemical constituents. This confirms and authenticates its use as malarial remedy in folkloric medicine.

Fever is one of the cardinal symptoms of malaria especially in humans. However, *P. berghei* infection in mice is reported to be associated with hypothermia rather than pyrexia [42]. Results of rectal temperatures of the infected mice in this study (curative test), showed that there was no significant difference between the mean temperature values of both the treated and untreated infected mice before and after treatment, suggesting that the mice were hypothermic. This hypothermia in mice may have resulted from the serious physiological effects of the malaria parasite on the host, leading to body heat loss and ultimately death of mice [43]. Moreso, carbohydrate, lipid, and protein metabolisms of the host are affected negatively by malaria parasite [44,45]. Decreases in metabolic rates of the *P. berghei*-infected mice have been correlated with decreased body temperatures of mice [46]. The extract/fractions however, were unable to attenuate these processes and hence the resultant hypothermia.

5. Conclusion

The results of this study show that the stembark extract and fractions of *Alchornea laxiflora* possess antimalarial potentials which maybe attributed to the activities of its phytochemical constituents.

Compliance with ethical standards

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Disclosure of competing interest

The authors have not declared any conflict of interests.

Statement of ethical approval

This study was approved and ethically cleared by College of Health Sciences Animal Ethics Committee, University of Uyo (UU/CHS/IHREC/2025/VOL.1/22)

Author's contribution

JEO, MUE - Research concept and design; JEO, MUE, GEE- Animal studies, JEO, UUF- Data analysis and interpretation; JEO, CCO, UUF- Writing the article. JEO, GEE, CCO and MUE read and approved the final manuscript.

References

[1] World Health Organisation. World Malaria Report 2024: Addressing inequity in the global malaria response. Geneva:World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO. https:// www. who.int/teams/global-malariaprogramme/reports/worldmalaria-report-2024;2024.

[2] Burkill HM. The useful plants of west tropical Africa 2nd Ed. Vol. 1, *Royal Botanic Garden* K. E.W. 960.;1994.

[3] Jain NK, Tailang M, Kumar S, Chandrasekaran B, Alghazwani Y, Chandramoorthy HC, Kumar A, Deshpande H, Wal P, Balamurugan M and Chidambaram K . Appraising the therapeutical potentials of *Alchornea laxiflora* (Benth.)

Pax & K.Hoffm., an underexplored medicinal herb: A systematic review. *Front. Pharmacol.* 2022; 13:958453. doi: 10.3389/fphar.2022.958453

[4] Farombi, E. O., Ogundipe, O. O., Uhunwangho, E. S., Adeyanju, M. A., and Moody, J. O. Antioxidant properties of extracts from *Alchornea laxiflora*(benth) Pax and hoffman. *Phytother. Res.*2003;17: 713–716.

[5] Oloyede GK. Onocha PA, Soyinka J, Oguntokun OW, Thonda E. Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. *Ann. Biol. Res.* 2010; 1; 114–120.

[6] Siwe-Noundou X, Ndinteh DT, Olivier DK, Mnkandhla D, Isaacs M, Muganza F M, Mbafor JT,Van Vuuren SF, Patnala S, Hoppe H, Krause RWM. Biological activity of plant extracts and isolated compounds from *Alchornea laxiflora:* Anti-HIV, antibacterial and cytotoxicity evaluation. *South African Journal of Botany* 2019;122 (2019) 498–503.

[7] Oyedeji AA, Angaye T CN,Abowei, J FN. Evaluation of *Alchornea cordifolia* Extracts for Larvicidal Activity against Anopheles. Implications for malaria Control. *UMYU Scientifica*, 2024;3(1):235 – 240.

[8] Elufioye TO. Memory-enhancing and memory-related beneficial effects of selected medicinal plants from the Nigerian flora," in Medicinal plants and fungi: Recent advances in research and development. Editors D. Agrawal, H. S. Tsay, L. F. Shyur, Y. C. Wu, and S. Y. Wang (Singapore: Springer), 2017;487–510.

[9] Mbaveng AT, Sandjo LP, Tankeo SB, Ndifor AR, Pantaleon A, Nagdjui BT, Kuete V. Antibacterial activity of nineteen selected natural products against multi-drug resistant Gram-negative phenotypes. *Springerplus* 2015; 4: 823.

[10] Okokon JE, Udoh AE, Osigwe CC, Uwaeme UF, Okon HT. (2025). Analgesic activity of ethanol stembark extract of *Alchornea laxiflora. Asian Journal of Biochemistry, Genetics and Molecular Biology*.17(6): 22-29.

[11] .Sandjo LP, Poumale HMP, Siwe XN, Ntede HN, Shiono Y, Ngadjui BT. Two new fatty acid derivatives from the stem bark of *Alchornea laxiflora (E*uphorbiaceae). *J. Americ. Oil Chem. Soc*. 2011; 88: 1153–1159.

[12] Tapondjou LA, Jeuett-Siems K, Siems K, Melzig M F. Alchornealaxine, an unusual prenylguanidinyl-epicatechin derivative from *Alchornea laxiflo*ra (benth) Pax and hoffman. *Rec. Nat. Prod*. 2016;10: 508–512.

[13] Trease GE, EvansWC. *Pharmacognognosy.* 15th Edition, Bailierr Tindall, 2002;pp. 683-684.

[14] Okokon, JE, Nwafor PA. Antiplasmodial activity of root extract and fractions of *Croton zambesicus*. *Journal of Ethnopharmacology.* 2009; 121:74-78.

[15] .Atanu FO, Idih FM, Nwonuma CO, Hetta HF, Alamery S, and Batiha GE. Evaluation of antimalarial potential of extracts from *Alstonia boonei* and *Carica papaya* in *Plasmodium berghei*-infected mice. *Evidence-Based Complementary and Alternative Medicine* Volume 2021, Article ID 2599191, 11 pages.

[16] Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archieves of Toxicology.* 1983; 54:275-286.

[17] Okokon JE, Antia BS, Azare B, Okokon PJ. (2017). Antimalarial and antiplasmodial activities of ethanol root extract of *Zea mays. Avicenna Journal of Phytomedicine.* 7(3):275-284.

[18] Okokon JE, Opara KN, Udobang JA, Bankhede H. (2019). *In vivo* antimalarial and antipyretic activities of leaf extract of *Ananas comosus*. *Tropical Journal of Natural Product Research*. 3(7): 240 -245.

[19] Okokon JE, Davis K,. Azare B, Okokon P. (2016). Analgesic and antimalarial activities of ethanol root extract of *Panicum maximum*. *African Journal of Pharmacology and Therapeutics*. 5(3): 128 -135.

[20] Okokon JE, Obot AU, Mittal G, Mohanakrishnan D, Sahal D. (2017b). Antimalarial and antiplasmodial activity of leaf extract of *Alchornea laxiflora. Journal of Herbs, Spices and Medicinal Plants.* 23(2):128-141.

[21] .Okokon, J.E., Augustine, N.B. and Mohanakrishnan, D. (2017a). Antimalarial, antiplasmodial and analgesic activities of root extract of *Alchornea laxiflora*, *Pharmaceutical Biology*, 55(1): 1022-1031.

[22] Waako, P. J., Gumede, B., Smith, P. and Folb, P. I. (2005). The *in vitro* and *in vivo* Antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* Schumch. et Thonn. *Journal of Ethnopharmacology*, 99(1):137-143.

[23] Philipson, J. D. and Wright, C. W. (1991). Antiprotozoal compounds from plants sources. *Planta Medica,* 57:553–559.

[24] Christensen SB, Kharazmi A. (2001). Antimalarial natural products. Isolation, characterization and biological properties. In: Tringali C, editor. Bioactive compounds from natural sources: isolation, characterization and biological properties. London: Taylor & Francis; p. 379–432.

[25] Kumaratilake LM, Robinson BS, Ferrante A, Poulos A. (1992). Antimalarial properties of n-3 and n-6 polyunsaturated fatty acids: *in vitro* effects on *Plasmodium falciparum* and in vivo effects on *P. berghei.* J Clin Investig. 89:961–967.

[26] Krugliak M, Deharo E, Shalmiev G, Sauvain M, Moretti C, Ginsburg H. (1995). Antimalarial effects of C18 fatty acids on *Plasmodium falciparum* in culture and on *Plasmodium vinckei petteri* and *Plasmodium yoelii nigeriensis in vivo*. *Expl Parasitol.* 81:97–105.

[27] Suksamrarn A, Buaprom M, Udtip S, Nuntawong N, Haritakun R, Kanokmedhakul S. (2005). Antimycobacterial and antiplasmodial unsaturated carboxylic acid from the twigs of *Scleropyrum wallichianum*. *Chem Pharm Bull.* 53:1327–1329.

[28] Attioua, B., Weniger, B., Chabert, P. (2007). Antiplasmodial activity of constituents isolated from *Croton lobatus*. *Pharmaceutical Biology.* 45:263–266.

[29] Melariri, P., Campbell, W., Etusim, P., Smith, P. (2011). Antiplasmodial properties and bioassay-guided fractionation of ethyl acetate extracts from *Carica papaya* leaves. *Journal of Parasitology Research,* Article ID 104954, 7pages.

[30] Melariri, P., Campbell, W., Etusim, P., Smith, P. (2012). In vitro and in vivo antimalarial activity of linolenic and linoleic acids and their methyl esters. *Advanced Studies in Biology,* 4:333–349.

[31] Attioua, B., Lagnikab, L., Yeoc, D., Antheaumed, C., Kaisere, M., Wenigerf, B., Lobsteinf, A., Vonthron-S_en_echeauf C. (2011). In vitro antiplasmodial and antileishmanial activities of flavonoids from *Anogeissus leiocarpus* (Combretaceae). *International Journal of Pharmaceutical Sciences Review and Research*, 11:1–6.

[32] Ganesh, D., Fuehrer, H., Starzengruber, P., Swoboda, P., Khan, W. A., Reismann, J. A., Mueller, M. S., Chiba, P., Noedl, H. (2012). Antiplasmodial activity of flavonol quercetin and its analogues in *Plasmodium falciparum*: evidence from clinical isolates in Bangladesh and standardized parasite clones. *Parasitology Research*, 110:2289–2295.

[33] Ezenyi, I. C., Salawu, O. A., Kulkarni, R., Emeje, M. (2014). Antiplasmodial activity aided isolation and identification of quercetin-40-methyl ether in *Chromolaena odorata* leaf fraction with high activity against chloroquine-resistant *Plasmodium falciparum. Parasitology Research*, 113:4415–4422.

[34] Kohno, Y., Egawa, Y., Itoh, S., Nagaoka, S., Takahashi, M., Mukai, K. (1995). Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in *n*-butanol. *Biochimica et Biophysica Acta.* 1256:52–56.

[35] Ponnamma, S. U., Manjunath, K. (2012). GC-MS analysis of phytocomponents in the methanolic extract of *Justicia wyaadensis* (NEES) T. Anders. *International Journal of Pharmaceutical and Biological Sciences* 3:570-576.

[36] Khan, S. L., Siddiqui, F. A. (2020). Beta-Sitosterol: As Immunostimulant, Antioxidant and Inhibitor of SARS-CoV-2 Spike Glycoprotein. *Archives of Pharmacology and Therapeutics*, 2(1):12-16.

[37] Cimanga RK, Tona GL, Kambu OK, Mesia GK, Muyembe JJ, Apers S. 2009. Antimalarial, antiamoebic and cytotoxic activities of some extracts and isolated constituents from the leaves of *Morinda morindoides* (Baker) Milne- Redh. (Rubiaceae). *Rec Progr Med Plants* 25:225–242.

[38] Lui KC, Yang SC, Roberts MF (1992). Antimalarial activity of *Artemisia annua* flavonoids from whole plants and cell cultures. *Plants Cell* II: 637–40.

[39] Teffo LS, Aderogba MA, Eloff JN (2010). Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscose* Jacq. Var. *angustifolia* leaf extracts. *South African Journal of Botany* 76:25–29.

[40] Kirmizibekmez H, Calis I, Perozzo R, Brun R, Donmez AA, Linden A, Ruedi P, Tasdemir D (2004). Inhibiting activities of the secondary metabolites of *Phlomis brunneogaleata* against parasitic protozoa and plasmodial enoyl-ACP reductase, a crucial enzyme in fatty acid biosynthesis. *Planta Medica.* 70:711.

[41] Ferreira JF, Luthria DL, Sasaki T, Heyerick A (2010). Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules.* 15:3135–3170.

[42] Belay, W. Y., Gurmu, A. E., Wubneh, Z. B. (2018). Antimalarial activity of stem bark of *Periploca linearifolia* during early and established *Plasmodium* infection in mice. *Evidence-Based Complementary and Alternative Medicine*. Volume 2018, Article ID 4169397, 7 pages.

[43] Jennings G, Elia M (1987). "Effect of *E. coli* endotoxin on temperature, oxygen consumption and brown adipose tissue thermogenesis in rats and mice," *Bioscience Reports,* 7(6): 517–523.

[44] Siddiqui WA, Trager W. (1967). Free amino-acids of blood plasma and erythrocytes of normal ducks and ducks infected with malarial parasite, *Plasmodium lophurae*," *Nature,* 214(5092):1046-1047.

[45] Angus MG, Fletcher KA, Maegraith BG. (1971).Studies on the lipids of *Plasmodium knowlesi*-infected rhesus monkeys (*Macaca mulatto*)I: Changes in serum lipids," *Annals of Tropical Medicine and Parasitology*. 65(2): 135–154.

[46] Briand, H., Peter, W. (1977) Acute malaria: effects of *P. berghei* on the metabolic rate of mice," *The Ohio Journal of Science,* 77– 189