International Journal of Biomedical and Clinical Sciences
Vol. 5, No. 3, 2020, pp. 229-235
http://www.aiscience.org/journal/ijbcs

In vivo Antimalarial Activity of Bark Extracts of *Lannea acida* (Anacardiaceae) and Chloroquine Against *Plasmodium berghei* in Mice

Olusegun Augustine Olusola, Isaiah Olabode Ogunsina*, Oluremi Samson Ayedogbon, Olufunmi Olusola Adesayo

Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Nigeria

Abstract

Lannea acida plants belong to the family Anacardiaceae. It has been used locally to treat and manage diseases that are contagious. Due to the recent increase in the resistance of malaria parasite to synthetic drugs the search for alternative treatment approach from plant sources is in urgent dimension. The antiplasmodial activity of mice was investigated with *L. acida* bark extracts. The study of the acute toxicity of the extract was used to determine lethal dose (LD_{50}). Suppressive activity of the extract was examined for five consecutive days with the extracts doses and chloroquine as the control drug, curative was infected for five days prior treatment, while the prophylactic groups were pretreated daily for five days before inoculation of 1×10^7 chloroquine-sensitive *Plasmodium berghei* infected erythrocyte intraperitoneally. Five mice were in each group of five groups. Whilst, the Control group was administered with 10 ml distilled water/kg; 100, 200, and 400 mg extract/kg weight were administered for the experimental groups, and chloroquine 5 mg/kg body weight respectively. All doses of the extract produced substantial, dose reliant, chemo suppressive activity against the parasite in the suppressive, curative and prophylactic tests as compared with chloroquine treated mice. A lengthen mean survival time of the treated mice was observed as compared to the untreated mice. The oral median lethal dose (LD_{50}) of the extract in mice was 5000 mg/kg body weight. Empirical results of the study showed that the *L. acida* bark extract is harmless. It has a potent antimalarial property which could be of future importance in malaria management and antimalarial drug design.

Keywords

Lannea acida, Antiplasmodial, *Plasmodium berghei*, Medicinal Plant

Received: May 11, 2020 / Accepted: June 15, 2020 / Published online: August 26, 2020

@ 2020 The Authors. Published by American Institute of Science. This Open Access article is under the CC BY license.
http://creativecommons.org/licenses/by/4.0/

1. Introduction

Malaria disease continues to be one of the most deadly parasitic diseases in human population. We are meant to know that malaria is the major causes of death in children under 5 years [1] An estimated population of 446,000 people dies per year [2], as a result of the ineffectiveness of the most used antimalarial drugs due to the resisting ability of the malaria parasite. In addition to the consequences of malaria on the health impact, the disease has a measurable direct and indirect cost, and has been known to be a major obstacle to socioeconomic development [3, 4].

In treating simple malaria throughout Africa which Nigeria is inclusive, Artemisinin Combination Therapy (ACT) is usually chosen first to combat malaria parasite. ACTs has been shown to pose high efficacy against plasmodium parasites and several have been shown to be moderately effective against the early stage of infection and reduce transmission to mosquitoes. Artemether-Lumefantrine (AL) is the most widely used ACT in Nigeria and Africa continent. The Dihydroartemisinin-piperaquine (DP) also possessed higher efficacy and its advantages of simpler dosage and a

* Corresponding author
E-mail address: metabolitebode@yahoo.com (I .O. Ogunsina)

longer prophylactic period [5].

[6] posited that a new global effort to eradicate malaria related diseases is to reduce the diffusion of the parasite. The prevalence of drug resistant to malaria parasites in prevalent area has posed a great threat to the usefulness and cheapest antimalarial drugs, chloroquine (CQ) and Sulphadoxine-pyrimethamine (SP). Presently, most malaria prevalent area in Africa including Nigeria have changed their first line antimalarial treatment from CQ or SP to artesunate/amodiaquine combination or artemether/lumefantrine combination. The ACTs used in utmost malaria prevalent countries have established high efficacy, protection against the development of resistance to each component and reduction of malaria transmission [7, 8]. However, malaria is often referred to as the disease of poverty and the cause of poverty, the relatively high of costs, dosage complexity and the limited experience of their use in sub-Saharan Africa has affected the widespread deployment of these drug combinations [9].

Lannea acida plants belong to the family (anacardiaceae) commonly called, awere Kogun in Akoko area of Ondo state Nigeria, Akogun in Ondo town and are used in locally in the control of contagious diseases majorly malaria. In hot and dry savannahs of sub Saharan Africa *L. acida* is one of the most dominant *Lannea* species. It has a rich history of ethnobotanical and ethno pharmacological usage in the management of several diseases which malaria is inclusive. We are meant to know that *L. acida* has significant medicinal applications. It has been documented that *L. acida* has been used in treating injuries, inflammation and pain, gastrointestinal challenges, fever and malaria, gynaecological and pregnancy disorders, ethnoveterinary medicine, haemorrhoids, skin diseases, and infections [10]. Stem bark extracts of *L. acida* are traditionally used in Nigeria as antiabortifacient, vermifuge and to treat anal haemorrhoids, diarrhoea, dysentery, malnutrition, and debility while the leaves is used to treat rheumatism. Information provided by the traditional healer in Akoko area of Ondo state revealed that the bark aqueous or alcoholic extract is used in the treatment of malaria. Even though *L. acida* demonstrated biological activity that validate their medicinal roles, no phytochemical studies were performed to isolate the chemical constituents responsible for the observed activity. On this note, the study seeks to x-ray the antiplasmodial action of the bark concentrate of *L. acida* in *Plasmodium berghei* impaired mice so as to give scientific proof to its consistent uses in the traditional mechanism of managing malaria.

2. Materials and Methods

2.1. Experimental Plant Material

The exterior part "bark" of *L. acida* plant was collected from Ugbe town, of Ondo state Nigeria and was authenticated at the Plant and Biotechnology Science Department of Adekunle Ajasin University Botanic Garden Herbarium. A sample specimen was stored at the herbarium for further use/or need. The *L. acida* concentration was dried by exposure to air in a shady place for twenty-one days and ground using a Wiley laboratory mill.

2.2. Extract Preparation

The bark of *L. acida* was allowed to dry at room temperature. They were pulverized in mechanized laboratory grinder (Manesty, England) to fine powder. The dried back weighing 500 g was soaked in 1 L of absolute methanol. The mixture was thoroughly mixed and filtered after 48 h using a Buchner vacuum filter. A rotary evaporator was used to dehydrate the filtered supernatant to anhydrous state. The weight of dried methanolic extract of the back was 47 g representing 9.4% yield in relation to the weight of the sample used. The work of [11] was used to get the percentage yield of the extract. The percentage yield is expressed as:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of ground plant material}} \times 100$$

2.3. Phytochemical Test

In conformation with [12], the screening of the methanolic extract was done to detect the existence of some ancillary metabolite such as saponin, tannin, alkaloids, terpenoids, steroid, Quinone, flavonoids and cardiac glycosides as discussed below.

2.3.1. Alkaloids Test

For 15 min, 5% ethyl ether was used to remove fat from the unrefined powder of 0.5 g. In a steam bath, with 5 ml of 1% of HCL (aq), the plant material that has been freed of fat was extricated for 20 min. A filter paper was used to sieve the solution. Adding ImL of Dragendorff's reagent to 1ml of filtrate, the solution was observed for changes in the colour to black or formation of precipitate. The changes in colour show that alkaloid is present.

2.3.2. Saponin Test

The presence of frothing is used to detect the presence of saponin in aqueous solution. In a test-tube, a 2 g of powder *L. acida* was dissolved in distilled water and shook the mixture. The continuous formation of bubble during warming of the solution was used as a prelude to detect that saponins are present.

2.3.3. Flavonoids Test

The work of [12, 13] was used to determine whether flavonoid is present in the extract. First, a dilute ammonia

solution of 5 ml was put into a quota of the aqueous filtrate of the extract. Second, conc H_2SO_4 was added. The observance of yellow colour shows that flavonoid is present.

2.3.4. Tannin Test

In line with Boma et al. (2016), 0.5 g of the powder sample of *L. acida* was boiled in 20 ml of distilled water in a test tube and in addition filtered, 0.1% of ferric chloride was added. The presence of a blue-black or brownish green colour shows that tannin is present [14].

2.3.5. Terpenoids Test: Salwowski Test

The test of salwowski was adopted here. An extract of 0.5 g was liquefied in 2 ml of chloroform [15], after which 3 ml of Conc. H_2SO_4 was added to form a layer. The presence of a reddish brown colour at the interface showed that terpenoids are present [15].

2.3.6. Steroids Test

To test for presence of steroid, methanolic extract was used. 0.5 g to 2 ml of acetic anhydrides with 2 ml of H_2SO_4 of methanolic extract was added. There is no colour generation indicating no presence of steroids.

2.3.7. Cardiac Glycosides Test

The Keller-Killani test is adopted here. A drop of 10% ferric chloride is mixed with 2 ml of glacial acetic acid to form a solution. Then, the extract (0.5 g) was diluted in the solution. Conc. Sulfuric acid (1 ml) is then added to the solution. This allows for layers to form beneath within the test tube that contains the solution. The attributes of deoxysugar of cardenolides is present when a brown ring at the interface appears. This means cardiac glycosides is present. Whilst, below the brown ring, a violet ring may show, a greenish ring may show slightly above the brown ring in the acetic acid layer and slowly cover more spaces within this layer [14, 16, 17].

2.3.8. Test for Quinone

The sample of the extract (0.2 g) was soften in distilled water and mixed with 1 ml of 10% NaOH, then shaken vigorously. Quinone is present when the solution showed blue, green or red colour.

2.4. Animals

The study used both sexes of Wistar mice (17-20 g), bred at the animal house of the institute of Advance Medical Research and Training (IAMRATS) Ibadan Oyo state Nigeria as the test animals. The animal ethics committee of Adekunle Ajasin University Akungba Akoko, Nigeria on the use of laboratory animals approved the use of both sexes of winster mice (17-20 g) for the study.

2.5. Acute Toxicity Study (LD 50)

The [18] deduced the method to determine lethal dose (LD50). The method was used for the study. Two stages were involved in the experiment:

Stage I: Nine mice were included in the first stage of the experiment. The mice were differentiated into three classes of three mice in every class. In Class A, methanolic concentrate of *L. acida* 10 mg/kg weight was dispensed to the mice. Mice in Class B were given one hundred mg/kg body weight. In Group C, the mice were dispensed one thousand mg/kg body weight. The mice were watched for twenty-four hours. The mortality in every class was recorded.

Stage II: The second stage of the experiment was done depending on the outcome of the previous stage. There are 3 classes of mice with each class having one mouse in every group. In Class A, the mouse was given 1600 mg/kg body weight of the methanolic concentrate of the plant extract. Whilst, the mouse in Class B was given 2900 mg/kg body weight, the mouse in Class C got 5000 mg/kg body weight of the methanolic concentrate of the plant extract. The mice were watched for additional twenty-four hours throughout that the mortality figure or anomalous response and odd attitude was recorded.

2.6. Rodent Parasite

Rodent inoculated with *P. berghei* that are sensitive to chloroquine was acquired from Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria, Oyo State, Nigeria. A standard inoculum of 1×10^7 of parasitized erythrocytes from a mice donor in volumes of 0.2 ml was used to inoculate the experimental animals. The re-infected mice were put at the animal house of Biochemistry Department of Adekunle Ajasin University Akungba Akoko where the study was done.

2.6.1. Early Test on Malaria Infection (4-day Suppressive Test)

In testing for early malaria infection, the work of [19] on 4day suppressive test against chloroquine sensitive *P. berghei* infection in mice was used as the basis for the study. In the study, twenty-five winster mice of both sexes were inoculated as early described. Random grouping of the mice was done. The resultant groups have five mice in each group. The mice received the extract every day for 4 sequential days. The treatment of the mice instantaneously follows the infection of the mice with the parasite. The control is the Group 1. The mice in the Group 1 were dispensed 10 ml/kg body weight of distilled water. Oral administration of extract was done in Groups 2, 3 and 4. The mice in Group 2, 3 and 4 received 100, 200, and 400 mg extract/kg body weight every day separately. The mice in Group 5 were given 5 mg

chloroquine /kg body weight orally every day. On the fifth day (D_5), blood was taken from the tail of every mouse. The collected blood samples were used to prepare a microscope thin film slide [20] method, the blood films were stained with Giemsa and observed with the microscope. The parasite count was recorded and the inhibition of parasitemia was communicated as a percentage for every dose, looking at the comparison of the parasitemia in the control group with the treated one.

$$\text{Average suppression} = \text{APC} - \text{APT}/\text{APC} \times 100.$$

Table 1. Qualitative phytoconstituents of *L. acida* bark extracts.

Sample	Saponin %	Flavonoids %	Alkaloids %	Terpenoids mg/100 g	Tannin mg/100 g	Phenol mg/100 g	Quinone %	Vitamin A mg/100 g	Vitamin C mg/100 g
Qualitative analysis	+++	+++	+++	++	+	+	+++	+++	+++

++/+++ = moderately present, + = slightly present.

Table 2. Curative effect of methanolic bark extract of *Lannea acida in P. berghei* infected mice.

Treatment		Mean parasitaemia count			Body Weight (g)		
Group	Dose (mg/kg	Pre-(D3)	Post-(D7)-Treatment	D7-Inhibition (%)	Survival Time Days) D1		D7
Control	N/S	17.2 ± 0.16	35.4 ± 0.4	0	6.8 ± 0.60	16.28 ± 0.06	15.2 ± 0.09
Extract	100	21.4 ± 0.22	16.2 ± 0.08**	54.31638	11 ± 1.42	17.42 ± 0.06	16.6 ± 0.05
Extract	200	16.4 ± 0.17	11.4 ± 0.26**	67.79096	20 ± 2.22	18.3± 0.05	16.98 ± 0.07
Extract	400	13.8 ± 0.08	6.4 ± 0.17**	81.57062	25.2 ± 1.81	17.34 ± 0.08	15.88± 0.12
Chloroquine	5	14.4 ± 0.1	1.4 ± 0.1**	96.04519	29.8 ± 0.08	15.48 ± 0.02	14.3 ± 0.06

D_3 = Day three, D_7 = Day seven, **significant different at $p<0.01$ compared to the negative control.
APC = Average parasitemia in the control.
APT = Average parasitemia in the test group.

2.6.2. Test on Convectional Infection (Rane Test)

The method formulated by [21] was used as the basis in assessing the curative potential of *L. acida* stem bark extract on infections that are common. Twenty-five mice were inoculated. The infected mice were untreated till the day four (D_4) after inoculation. The mice were weigh and randomized into five groups of five mice per group. Mice in Group 1 were given 10 ml/kg of distilled water. Oral administration of the extract was done in Group 2, 3 and 4. Specifically, 100, 200 and 400 mg extract /kg body weight were dispensed to the mice in group 2, 3, and 4. Mice in Group 5 received 5mg chloroquine/kg body weight orally for four days consecutively (D_4-D_7). On day 7, blood was gathered from the tail of every mouse and a thin blood film was made on a microscopic slide surface. The blood films were stained with Giemsa stain. The stained blood films were observed with the microscope to determine the parasitemia level. The mean survival time of the mice in every tested group was recorded for thirty days [22, 23].

2.6.3. Repository Test

The residual infection method formulated by [19]. The methods were used to estimate the prophylactic ability of the *L. acida* stem bark extract. Twenty-five mice of both sexes were weighed. The 25 mice were randomly differentiated into 5 groups with 5 mice in every group. Mice in group 1 were given 10 ml/kg distilled water. Oral administration of the extract was done in group 2, 3 and 4. Specifically, 100, 200 and 400 mg extract /kg body weight were dispensed to the mice in group 2, 3, and 4. Mice in Group 5 received 5 mg chloroquine/kg body weight orally every day consecutively. Continuous treatment of the mice was done every day for four days (D_1-D_4). On the 5th day, all the mice were infected with the parasite. On day 7 (D_7) blood films was prepared from every mouse 72 h post treatment [24]. The blood films were observed with the microscope to determine the mean parasitemia in every group. The body weight of the mice was re-weighed on day 7, and the differences between the before- and after-treatment body weight were recorded.

2.7. Statistical Analysis

Results obtained were analysed using graph pad prism version 7. The results were reports as mean ± standard error of mean. The differences between means were analysed and compared using two-way ANOVA, followed by Dunnet's test. The significance value ($P< 0.01$) was chosen as significant level.

3. Results

The phytoconstituents of the experimental plant is depicted in Table 1 and the antiplasmodial evaluation is depicted in Table 2 Figures 1 and 2.

From the acute toxicity tests of the crude methanolic extract following oral administration and using Lorke's modified method, the LD_{50} was calculated to be 5000 mg/kg (5 g/kg). An apparently high LD_{50} obtained from this study suggest that the extracts are very safe in mice and rats when administered orally.

4. Discussion

The present outcomes from the study demonstrated that the bark extract of *L. acida* have a huge suppressive impact against early *Plasmodium* infection, curative impact against conventional infection and prophylactic impact against residual infection in *P. berghei infected* mice. The acute toxicity of *L. acida* bark methanolic extract was tested orally in mice following Lorke's technique [18]. The mice survival, after oral administration of 3500 mg/kg body weight of the extract, up to seven days, indicates that the predictable oral median lethal dose (LD_{50}) of the extract at 5000 mg/kg body weight is nontoxic. This indicates that acute oral usage of the extract is harmless, and also justify why the bark portion of the plant is widely used locally in the management of malaria.

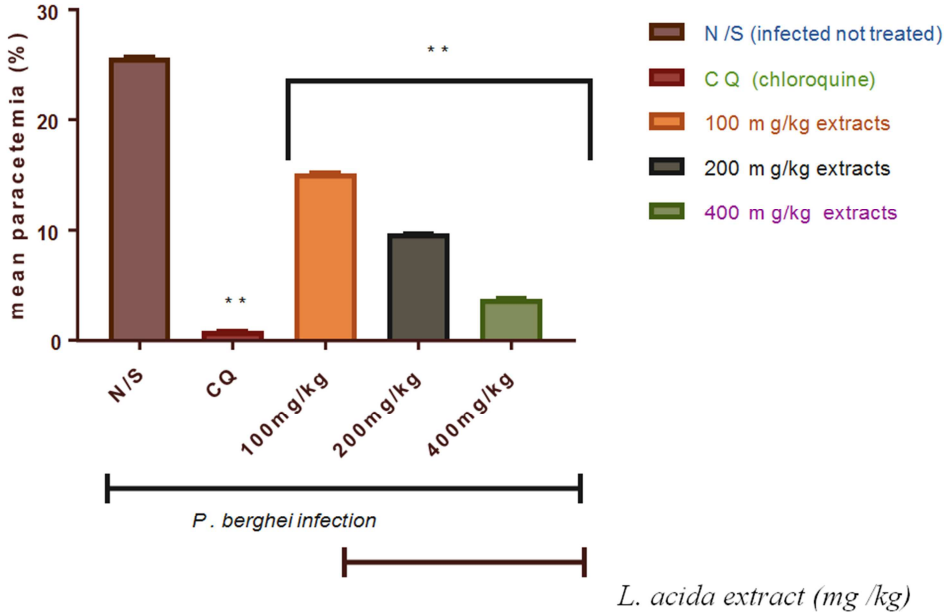

Figure 1. Suppressive activity of methanolic bark extract of *Lannea acida in P. berghei* infected mice. **Significant different at $p< 0.01$ compared to the negative control.

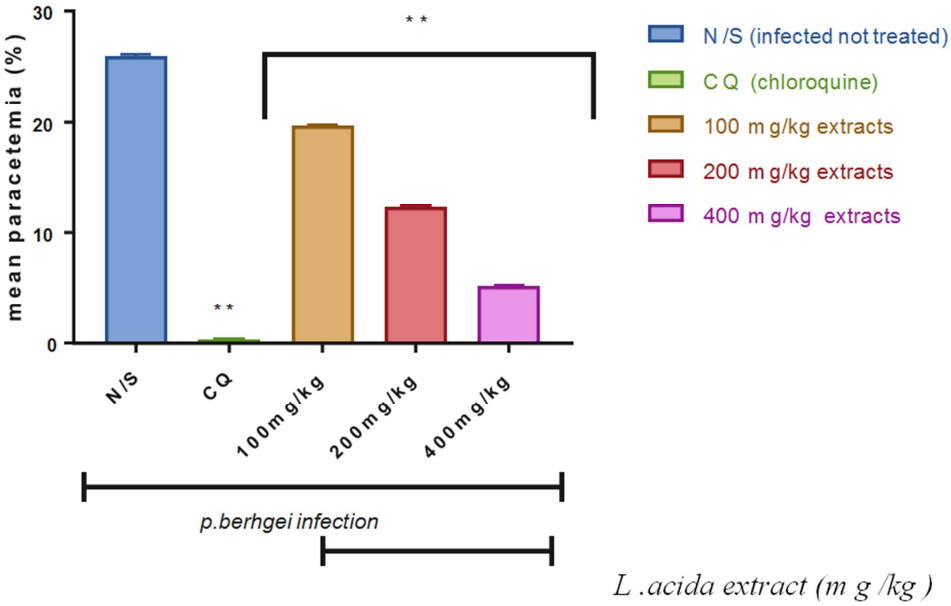

Figure 2. Prophylactic effect of methanolic bark extract of *L. acida in P. berghei* infected mice. **Significant different at P<0.01 compared to the negative control.

Meanwhile mice model does not precisely give the exact indications detected in the human plasmodium infection but they have been similar disease symptom to human plasmodium infections when infected with *P. berghei*, which have been documented by [25, 26]. The use of *P. berghei* in evaluating the therapeutic outcome of assumed malaria strapping drugs in many studies [27, 23] validate its usage in this study. *P. berghei* is highly sensitive to chloroquine,

making it suitable for this study. Constituents that bring about reduction in parasite multiplication in the host cell were considered to possess antimalarial activity [21].

The early malarial investigation is a regular assessment usually used for antimalarial study [19]. In all the treated groups the results shown substantial dose reliant chemo suppression (85.27%) shown in the group treated with 400 mg of the sample concentrate/kg btw, followed by 78.29 and 68.21% for 200 and 100 mg/kg respectively, while chloroquine drug is 98.44% respectively (Figure 1). The methanolic bark extract of *L. acida* also established significant dose dependent decrease in parasite count in both curative and prophylactic infection, similar to the result of chloroquine (Table 2 and Figure 2), which is an established antimalarial drug. The decrease in parasite count in the curative test is similar to the values observed in parasite count of the suppressive test, but lower than the mean parasite counts in the repository test. This may be as a result of prompt parasite clearance by the extract in early and established infection, as against a situation where the extract was mostly administered for days.

(Prophylactic) before inoculation with parasite. The result conformed with [28] which established that high parasite count in the repository test may be ascribed to speedy metabolism of administered extract to inactive products. The significant reduction in body weight in the curative and prophylactics group administered with extracts as well as the control group may be due to combined effect of plasmodium infection [29] and possible catabolic effect of the doses of the extract on the stored lipids. These observations indicate that the extract is potent against the malaria parasite used in this study and is consistent with the ethno medicinal use of *L. acida* plants, as antimalarial in Northern part of Nigeria [30].

The mechanism of antiplasmodial activities of the *L. acida* extract has not been elucidated, however, antiplasmodial effects of natural plant products have been credited to some of their active phytochemical components [16]. Some of these phytochemicals such as terpenoids, quinone, saponin, alkaloids and flavonoids detected in *L. acida* might contribute to its antiplasmodial activity. The antiplasmodial effect of bark extract of *L. acida* may therefore be due to the phytochemical components (alkaloids, flavonoids, quinone, saponin and terpenes) or the oxidant generation potential or a combination of these mechanisms.

5. Conclusion

The outcomes of this study show that the bark of *L. acida* plants possesses moderate antimalarial activity in mice. This result has established the rationale for the traditional use of the *L. acida* in the treatment of malaria. The study agrees with the common saying that plants that have high therapeutic value in treating malaria should be studied scientifically so as to ascertain their efficacy and potentials as sources of new antimalarial drugs.

Authors' Contributions

Ogunsina and Olusolas performed the experiments while the manuscript was written by Ogunsina but edited by Olusola. Ogunsina analysed and discussed the data. All the authors designed the study and reviewed the manuscript, read and approved the final manuscript.

Funding

This research was funded from personal contributions by the authors.

Availability of Data and Materials

The data sets analysed in this current study are available from the corresponding author on request.

Ethics Approval and Consent to Participate

This study was approved by Adekunle Ajasin university Animal laboratory handling Committee.

Consent for Publication

Not applicable.

Competing Interests

The authors declared that there is no competing interest.

Declaration of Conflicting Interests

The author (s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgements

We are grateful to God for His support at all times.

References

[1] World Health Organization (WHO) (2008). World Malaria Report, pp. 7-10. https://www.who.int/malaria/publications/atoz/9789241563697/en/ World Health Organization.

[2] (WHO) (2016). World Malaria Report, pp. 13-15. https://www.who.int/malaria/publications/world-malaria-report2016/report/en/.

[3] Roll Back Malaria (RBM, 2001). Roll Back Malaria Partnership: Malaria in Pregnancy. World Health Organization, 20 Avenue Appia, CH-1211 Geneva 7, Switzerland. www.rollbackmalaria.organisation...06/12/2010.

[4] Sachs J, Malaney P (2002). The economic and social burden of malaria. Nature 415: 680-685.

[5] Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, Menéndez C, Nambozi M, Valéa I, Nabasumba C, Sasi P, Bacchieri A (2009). Dihydroartemisinin- piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: A randomised, non-inferiority trial. PLoS One 4 (11): e7871.

[6] Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F, Doumbo OK, Greenwood B, Hall BF, Levine MM, Mendis K (2011). A research agenda to underpin malaria eradication. PLoS Medicine 8 (1): e1000406.

[7] Bloland PB, Ettling M, Meek S (2000). Combination therapy for malaria in Africa: Hype or hope? Bulletin of the World Health Organization 78: 1378-1388.

[8] Sutherland CJ, Ord R, Dunyo S, Jawara M, Drakeley CJ, Alexander N, Coleman R, Pinder M, Walraven G, Targett GA (2005). Reduction of malaria transmission to Anopheles mosquitoes with a six-dose regimen of co-artemether. PLoS medicine 2 (4): e92.

[9] Bloland PB, Kachur SP, Williams HA (2003). Trends in antimalarial drug deployment in sub-Saharan Africa. Journal of Experimental Biology 206: 3761-3769.

[10] Maroyi A (2018). *Lannea acida*: A review of its medicinal uses and Phytochemistry and pharmacological properties. Asian Journal of Pharmaceutical and Clinical Research 11: 11.

[11] Banso A, Adeyemo S (2006). Phytochemical screening and antimicrobial assessment of Abutilon mauritianum, Bacopa monnifera and *Datura stramonium*. Biokemistri 18: 39-44.

[12] Sofowora A (1993). Medicinal plants and traditional Medicine in Africa. (2nd Edition), Spectrum Books limited. Sunshine House Ibadan, Nigeria. pp. 134-156.

[13] Harborne JB (1993). Phytochemistry Academic Press, London. pp. 89131.

[14] Trease G. E., and Evans W. C. (1989). Pharmacognosy, 11th edition. *Bailliere Tindall, London* pp. 45-50.

[15] Boma O. T, Worlu-Wodo Q. E, Deekae S (2016). Bioprospective Screening of *Ricinodendron Heudelotii* seeds. Journal of Analytical and Pharmaceutical Research 3 (7): 00084.

[16] Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. Tropical Journal of Pharmaceutical Research 7 (3): 1019-1024.

[17] Okhale SE, Ugbabe EG, Bamidele O, Ajoku AG, Egharevba HO (2014). Phytochemical and antimicrobial studies on extractives of *Calyptrochilum emarginatum* (SW) Schltr (Orchidaceae) growing in Nigeria. Journal of Medicinal Plants Research 8 (4): 223-228.

[18] Lorke D (1983). A new approach to practical acute toxicity testing. Archives of Toxicology 54: 275-287.

[19] Peters W (1965). Drug resistance in *plasmodium berghei*. Vinka and Lips. Experimental. Parasitology., 17: 80-89.

[20] Chees brough M (2004). District Laboratory Practice in Tropical Countries. 2nd ed. Cambridge University Press pp. 239-258.

[21] Ryley JF, Peters W (1970). The antimalarial activity of some quinolone esters. Annals of Tropical Medicine and Parasitology 64: 209-222.

[22] Saidu K, Onah J, Orisadipe A, Olusola A, Wambebe C, Gamaniel K (2000). Antiplasmodial, analgesic and antiinflammatory activities of the aqueous stem bark of *Erythrina senegalensis*. Journal of Ethnopharmacology 71: 275-280.

[23] Adzu B, Salawu OA (2009). Screening *Diospyros mespiliformis* extract for antimalarial potency. International Journal of Biological and Chemical Sciences 3 (2): 271-276.

[24] Abatan MO, Makinde MJ (1986). Screening *Azadirachta indica* and *Pisum sativum* for possible ant malarial activities. Journal of Ethnopharmacology 17: 85-93.

[25] Pedroni HC, Bettoni CC, Spalding SM, Costa TD (2006). *Plasmodium berghei:* Development of an irreversible experimental malaria model in Wistar rats. Experimental Parasitology 113: 193-196. Peters W (1965). Drug resistance in *plasmodium berghei*. Experimental Parasitology 17: 80-89.

[26] Pierrot C, Adam E, Laffite S, Godin C, Dive D, Capron M, Khalife J (2003). Age related susceptibility and resistance to *Plasmodium berghei* in mice and rats. Experimental Parasitology 104: 81-85.

[27] Agbedahunsi JM (2000). Screening of crude drugs for the treatment of malaria in Nigeria, Phytomedicine in malaria and sexually transmitted diseases: Challenges for the new millennium. Drug Research and Production Unit, Faculty of pharmacy, Obafemi Awolowo University, Ile Ife, Nigeria. pp. 13-22.

[28] Dahanukar SA, Kulkarni FA, Rege NN (2000). Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology 32: 1081-118.

[29] Thomas AM, Van Der Wel AM, Thomas AW, Janse CJ, Waters AP (1998). Transfection system for animal models of malaria. Parasitology Today 14: 248-249.

[30] Etkin NL (1997). Antimalarial plants used by Hausa in Northern Nigeria. Tropical Doctor 27: 12-16.