

ORIGINAL RESEARCH ARTICLE

Antimalarial potential of five Nigerian medicinal plants: Repository versus curative activities

Funmilayo I. D. Afolayan^{1*}, Olayemi Adegbolagun², Beatrice Irungu³, Jennifer Orwa³, and Chiaka Anumudu¹

¹Department of Zoology, University of Ibadan, Ibadan, Nigeria

²Department of Pharmaceutical Chemistry, University of Ibadan, Ibadan, Nigeria

³Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, Nairobi, Kenya

Abstract

The conventional management of malaria in most endemic areas is based on phytomedicine. It is commonly believed that prevention is better than cure when it comes to disease management. Therefore, medicinal plants commonly used for treatment in herbal medicine are also used for prevention purposes. Hence, it is important to investigate the efficacy of medicinal plants in relation to the timing of their use. To document the medicinal plants used for treating malaria, a structured questionnaire-based ethnobotanical survey was conducted in Omu-Aran, Kwara State, Nigeria. The survey revealed the use of 31 plant species from 24 families, with origin in Omu-Aran, in herbal antimalarial recipes. Some of the identified plants were subjected to *in vivo* antimalarial bioassays. The aqueous and dichloromethane: Methanol (1:1) extracts of the leaves and stem bark of *Morinda lucida* and *Nauclea latifolia*, as well as the leaf extracts of *Chromolaena odorata*, *Tithonia diversifolia*, and *Lawsonia inermis* were tested at doses of 100, 250 and 400 mg/kg against *Plasmodium berghei* Anka. The repository and curative tests were conducted to assess the residual and curative abilities of the extracts, respectively, with chloroquine as a reference drug. The tested extracts demonstrated better antiplasmodial activities in the repository tests, particularly the aqueous extracts. Only the organic extract of *T. diversifolia* at 100 mg/kg exhibited a high antiplasmodial activity with a percentage chemosuppression (PCS) value of 66.13%, while other extracts showed moderate (PCS: 30 – 60%) to no activity (PCS: <0%) in curative tests. In repository tests, only *N. latifolia* showed high activity with percentage chemoprophylaxis (PCP) values ranging from 61.51% to 81.69%, while other extracts generally showed moderate activities. Chloroquine showed strong chemosuppression ($92.74 \leq \text{PCS} \leq 98.77$) in curative tests but weak chemoprophylaxis (PCP <30%). The efficacy tests showed that most of the investigated medicinal plants were more effective for prevention rather than for curative purposes.

Keywords: Medicinal plants; Antiplasmodial; Curative test; Repository test; Chloroquine

*Corresponding author:

Funmilayo I. D. Afolayan
(fidifede@gmail.com)

Citation: Afolayan AI, Adegbolagun O, Irungu B, *et al.*, 2023, Antimalarial Potential of Five Nigerian Medicinal Plants: Repository versus Curative Activities. INNOSC Theranostics and Pharmacological Sciences, 6(2):0300.
<https://doi.org/10.36922/itps.0300>

Received: February 28, 2023

Accepted: June 30, 2023

Published Online: July 26, 2023

Copyright: © 2023 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1 Introduction

The global impact of malaria on human health and social well-being is detrimental, with particular emphasis on its effect on children under the age of five. According to

the World Health Organization (WHO) in 2022, this specific age group accounted for 77% of all malaria-related deaths worldwide^[1]. In 2020, the number of malaria cases increased to 241 million, an increase from 228 million cases in 2018, with 627,000 of these cases resulting in fatalities. Notably, 96% of these deaths occurred in the African region^[1]. The WHO identified twenty countries in the African region, along with India, as the locations where approximately 85% of all global malaria deaths occur.

On the global scale, Nigeria bears a very high malaria burden, with the highest cases of malaria-related death (24%), followed by the Democratic Republic of Congo, with an estimated occurrence of about 11% of malaria-related death. The economic and social effects of malaria should not be overlooked, as it leads to a reduction in the Gross Domestic Product of Nigeria by approximately 1% annually and is the primary cause of absenteeism^[2].

The increasing incidence of malaria, a rise in malaria-related death, and the growing resistance of the malaria parasite to synthetic drugs have sparked interest in alternative treatment strategies. Traditional medicinal plants have historically played a significant role in the development of novel antimalarial drug therapies. For instance, compounds such as quinine and its derivatives are primarily derived from the *Cinchona* plant, while artemisinin is derived from *Artemisia annua*^[3,4]. These discoveries highlight the potential of medicinal plants to provide valuable insights into effective anti-parasitic compounds. Consequently, it is important to properly document and scientifically investigate the diverse range of medicinal plants that abound in Nigeria to validate their traditional usage.

Various medicinal plants folklorically used for the treatment of malaria have been scientifically confirmed. Some of these plants include *Chromolaena odorata*, *Nauclea latifolia*, *Lawsonia alba lam.*, *Morinda lucida*, and *Tithonia diversifolia*.

C. odorata (L.) is a perennial shrub that belongs to the *Asteraceae* plant family. In Nigeria, the plant is known for its antimicrobial and wound-healing properties and is commonly used in the treatment of various ailments, such as diarrhea, malaria fever, toothache, diabetes, skin diseases, dysentery, and colitis^[5,6].

N. latifolia Smith (*Rubiaceae* family) is a plant used in Africa for treating various ailments, including malaria^[7,8].

Lawsonia inermis L. (*Lythraceae* family), commonly called Henna, is widely spread and cultivated in North Africa, Australia, Asia, and the Northern and Southern parts of Nigeria^[9,10]. According to the World Flora Online (WFO) database, *L. inermis* is synonymous with *Lawsonia*

speciosa L., *Lawsonia purpurea* Lam., *Lawsonia coccinea* Sm, *Lawsonia alba* Lam., and *Lawsonia spinosa* L. (WFO, 2022)^[11]. Its plant parts, including the leaves, flowers, seeds, stem bark, and roots, have been reported to exhibit many biological activities, which include wound healing, antimicrobial, antioxidant, anticancer, antidiabetic, antimalarial, hepatoprotective, and hypoglycemic^[12-14].

M. lucida (L.), also commonly called the Brimstone tree, is a medicinal plant that belongs to the family of *Rubiaceae* and is located in the tropical West African rainforest^[15]. Conventionally, the leaves are used as an analgesic, antipyretic, laxative, and antimalaria^[16]. Different parts of the plant have also been reported to possess strong trypanocidal, aortic vasorelaxant, anticancer, hepatoprotective, antispermato-genic, anti-inflammatory, hypoglycemia, and antidiabetic activities^[14,17-19].

T. diversifolia (Hemsl.) A. Gray, commonly called the Mexican sunflower, belongs to the family of *Asteraceae*. It is used folklorically to treat ailments, including malaria. It has scientifically been reported to possess antiplasmodial activity^[13].

This report focuses on the ethnobotanical survey conducted in Kwara State, Nigeria, to identify local plants used to treat malaria. In addition, it presents the *in vivo* antiplasmodial activity of aqueous and organic extracts of leaves of *T. diversifolia*, *C. odorata*, and *L. inermis*, as well as the leaves and stem bark extracts of *N. latifolia* and *M. lucida*. The present study investigated the effects of administration timing of these extracts on mice infected with *Plasmodium berghei* ANKA strain, a rodent malaria parasite.

2 Materials and methods

2.1. Description of the study area

Omu-Aran is the headquarter of Irepodun Local Government Area, one of the 16 Local Government Areas in Kwara State. It is located at coordinates 8°2'17" N, 5°10'30" E at 80 km from the state capital, Ilorin. The region is home to Igbomina ethnic group, and the major spoken language is Yoruba (Figure 1).

2.2 Ethnobotanical data collection

The ethnobotanical survey was conducted among traditional healers and users of traditional medicine. Socio-demographic data of respondents were collected. Ethnobotanical data were collected mainly through oral interviews using a structured questionnaire. Information on local plant names, useful plant parts, preservation of plant materials, methods of preparation, application mode, dosage, and duration of treatment were recorded during

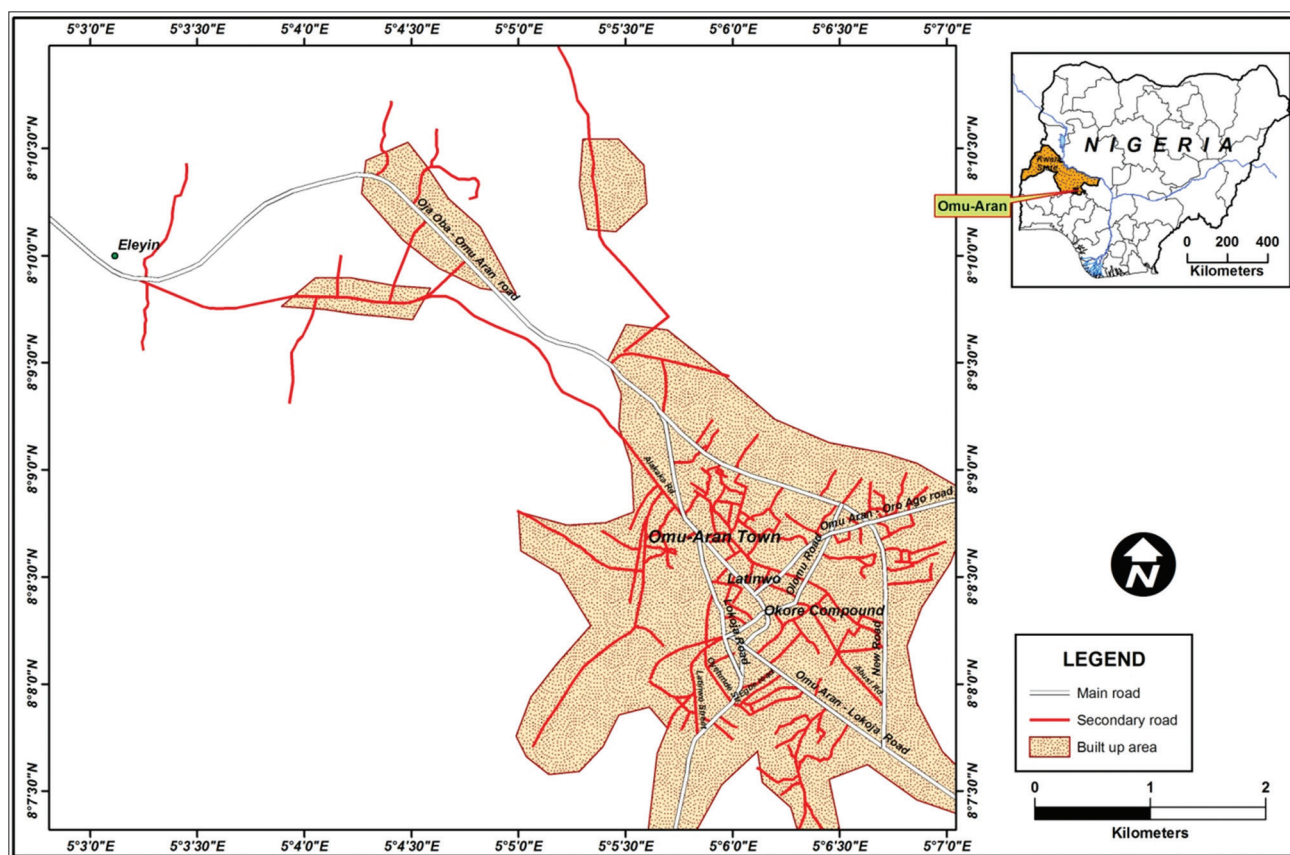


Figure 1. Map of the study site, Omu-Aran, Kwara State, Nigeria.

the interviews and the plants' names were validated and verified with information available on the website (<http://www.theplantlist.org>).

2.3. Collection, authentication, processing, and extraction of plant materials

Fresh leaves of *T. diversifolia*, *C. odorata*, and *L. inermis* and stem bark and leaves of *M. lucida* and *N. latifolia* were collected, identified, authenticated, processed, and extracted as previously described in^[9,10]. The following voucher numbers were given during the authentication and identification of the plants at the Forestry Research Institute of Nigeria: FNI 108427 (*C. odorata*), FNI 108428 (*T. diversifolia*), FNI 108429 (*L. inermis*), FNI 108430 (*N. latifolia*), and FNI 108431 (*M. lucida*).

2.4. Experimental animals

In this study, 6 – 8 weeks old healthy Swiss albino mice, weighing 20 ± 2 g, were obtained from Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. The mice were fed on commercial rodent food and water *ad libitum* in standard Makrolon type II cages in air-conditioned rooms at 22°C and 50 – 70% relative humidity, complying with the internationally accepted principles for laboratory

animal use and care as found in the WHO guidelines. The Animal Care and Use Committee (ACUC) guidelines of KEMRI were followed.

2.5. Parasite species, preparation of inocula, and inoculation of animals

Chloroquine-sensitive *P. berghei* ANKA strain was used for *in vivo* antiplasmodial study. The parasite was obtained from the Centre for Traditional Medicine (CTMDR), KEMRI, Nairobi, Kenya. The parasite was maintained through weekly passaging. The preparation of inocula and inoculation of animals were carried out according to a previous study^[13].

2.6. Experimental design and drug administration

The assay protocols used were Rane's (curative) and repository (prophylactic) tests, as previously described^[20-22]. A total of 25 mice per test (curative and repository) were used for the study. In each test, the mice were inoculated with 0.2 mL of blood containing 1.2×10^7 parasitized red blood cells. The animals were divided into five groups ($n = 5$). Three of the groups were orally administered 100 mg/kg, 250 mg/kg, or 400 mg/kg of the extract with the aid of a cannula, and the other two groups were the

negative and positive controls which were given the vehicle (tween 80/distilled water) and chloroquine diphosphate, respectively. Mice were treated with the extracts, vehicle, and standard drug once daily, and the level of parasitemia was examined following the treatment. The animals were given their doses of extract on day 4 after the inoculation of parasites on D_0 , then once daily for 2 more days ($D_4 - D_5$) for the curative test and before inoculation for the repository test.

2.7 In vivo plasmodial bioassay

2.7.1 Evaluation of antimalarial properties of the extract on established infection (curative test)

The curative potential of the test plants was evaluated using the method described in a previous study^[21]. Twenty-five mice were divided into five groups ($n = 5$). Each mouse was inoculated intraperitoneally on the 1st day (D_0) with infected blood containing *P. berghei*-parasitized red blood cells and left untreated. On day 3 (D_2) of the experiment, a pretreatment blood smear of each mouse was made to confirm the infection. Animals were treated daily for 3 days ($D_3 - D_5$). Blood smears were collected on day 7 post-infection (D_6), the percentage of parasitemia was determined, and the percentage chemosuppression (PCS) was calculated using the formula below:

$$A = \frac{B - C}{B} \times 100 \quad (1)$$

where:

A=Average PCS, B=Average percentage of parasitemia in the negative group, and C=Average percentage of parasitemia in the test group^[23].

2.7.2 Evaluation of antimalarial properties of extract on the residual infection (repository test)

The residual infection protocol was a modification of the procedure described in a previous study^[24] to evaluate the prophylactic activity of the extract. A set of mice in various groups categorized as above were used. Animals were pre-treated before infection for 3 days. Treatment was initiated on D_0 and continued till D_2 , and inoculated on D_3 with the parasite. On days 3 and 5 (D_6 and D_8) post-infection, blood smears were obtained from each mouse. The percentage of parasitemia and chemoprophylaxis were then determined. The chemoprophylaxis was also calculated using Equation 1.

2.7.3 Evaluation of parasitemia

Thin blood films were prepared by collecting blood from the tail of each mouse and smeared on a properly cleaned and well-labeled microscopic slide. The thin blood films were fixed in methanol, stained with 10% Giemsa at pH 7.2

for 20 min, and then microscopically examined ($\times 100$ magnification).

2.8 Statistical analysis

The results were expressed as mean $\pm$ SD. Descriptive statistics were used to present the results and Student's *t*-test was used to compare the treated and negative control groups.

3 Results

3.1 Medicinal plants used in the management of malaria in Omu-Aran

A total of 22 respondents were interviewed: Two traditional healers and twenty individual users of herbal medicine. They were aged between 34 and 91 years, and their knowledge of herbal medicine was acquired from relatives, spouses, parents, and friends. The ethnobotanical survey revealed a total of 31 plant species belonging to 24 plant families, with the highest number of antimalarial plants from *Rubiaceae*, followed by *Asteraceae*, *Anacardiaceae*, *Poaceae*, and *Musaceae* (Table 1).

The leaf was the most frequently used part of the plant for medicinal preparations, followed by the stem barks, while two to three parts of the same plant are sometimes used. Medicinal plants were dried and kept aerated until used. Some respondents indicated that the lack of moisture content in the dried forms facilitates the preservation process, which they considered superior to using fresh materials.

The medicinal plants were prepared using various methods, including boiling the material in water (decoction), soaking the plant material in water (infusion), or soaking the plant in alcohol, aqueous extract from fermented maize (pap water), or other solvents such as a carbonated drink or non-alcoholic beverages (tincture). It was indicated that most of these medicinal plants are consumed orally in the form of decoction. For specific cases, steam baths and oral consumption of remedies were recommended, particularly when the materials were prepared with decoction.

According to the respondents, all medicinal plants were considered collectible throughout the year and at any time during the day, with the exception of the period between 6 pm and 7 am. In addition, many respondents indicated that they avoided collecting plants on the town's market days due to their superstitious beliefs. The collection of plants was not associated with any specific manner or the need for incantations.

The dosage for most of the remedies was usually half of a small glass cup or stainless cup (approximately 100 mL),

Table 1. Plants used in the management of malaria in Omu-Aran, Nigeria

Species name	Family	Common name	Local name	Part used
<i>Tithonia diversifolia</i> (Hemsl) A. Gray	Asteraceae	Tree marigold	June 12/Jogbo	Leaves
<i>Chromolaena odorata</i> (L.) R.M king & H. Rob	Asteraceae	Siam weed	Akintola	Leaves
<i>Magnifera indica</i> J.F.Macbr	Anacardiaceae	Mango	Mongoro	Leaves and stem bark
<i>Lawsonia inermis</i> var. <i>spinosa</i> (L.) pers.	Lythraceae	Henna plants	Laali	Leaves
<i>Azadirachta indica</i> A. Juss	Meliaceae	Neem	Dongoyaro	Leaves and stem bark
<i>Psidium guajava</i>	Myrtaceae	Guava	Gilofa	Leaves
<i>Carica papaya</i> L.	Caricaceae	Pawpaw	Ibepe	Leaves, root, fruits
<i>Gossypium barbadense</i> L.	Malvaceae	Cotton	Owu	Leaves
<i>Senna siamea</i> (Lam.) H.S irwin and Barneby	Caesalpiniaceae	Senna	Kasia	Leaves and stem bark
<i>Citrus aurantifolia</i> f. <i>aurantifolia</i> (christm) M.Hiroe	Rutaceae	Lime	Osanwewe	Leaves and fruits
<i>Alstonia boonei</i> De wild	Apocynaceae	Stool wood	Ahun	Root, stem bark and leaves
<i>Anacardium occidentale</i> L.	Anacardaceae	Cashew nut tree	Kasu	Leaves
<i>Cymbopogon citratus</i> stap f.	Poaceae	Lemon grass	Ewe tea	Leaves
<i>Enantia polycarpa</i> Engl.&Diels	Annonaceae		Dokita Igbo	Stem bark
<i>Aloe vera</i> (L.) Burnm. F	Aloeaceae	Aloe	<i>Aloe vera</i>	Gel
<i>Ocimum basilicum</i> subsp. <i>gratissimum</i>	Labiatae	Sweet and hairy basil	Ejinrinwewe	Whole plant
<i>Axonopus compressus</i> P. Beauv.	Poaceae	Tropical carpet grass	Idi	Leaves
<i>Cocos nucifera</i> L.	Palmae	Coconut palm	Agbon	Shaft
<i>Ananas comosus</i> (L.) Merr.	Bromeliaceae	Pineapple	OpeOyinbo	Unripened fruit
<i>Nauclea latifolia</i> Blanco	Rubiaceae	Nauclea African peach	Ogbesi	Leaves, stem bark and root
<i>Nauclea diderrichii</i> merr.	Rubiaceae	Opepe African peach	Pepe	Leaves
<i>Khaya grandifoliola</i> A.Juss	Maliaceae	African Mahogany	Oganwo	Stem bark
<i>Morinda lucida</i> A.Gray	Rubiaceae	Brimstone tree	Oruwo	Leaves and stem bark
<i>Allium sativum</i> L.	Liliaceae	Garlic	Ayuu	Bulb
<i>Kigelia africana</i> (Lam.) Benth.	Bignoniaceae	Sausage tree	Pandoro	Leaves
<i>Aldama excelsa</i> (wills) E.E school.&panero	Moraceae	Iroko	Iroko	Stem bark and leaves
<i>Corchorus olitorus</i> L.	Tiliaceae	Jute plant	Ewedu	Leaves
<i>Ficus opposita</i> Car.opposita now.	Labiatae	Hoshindia	Efinrin	Whole plant
<i>Musa paradisica</i> L.	Musaceae	Plantain	OgedeAgbagba	Leaves, roots, fruit
<i>Musa sapientum</i> L.	Musaceae	Banana	Ogedeomini	Withered leaves
<i>Vernonia amygdalina</i> Del.	Compositae	Bitter leaf	Ewuro	Leaves

and the duration of treatment usually lasted for 3 – 5 days or until the symptoms disappeared. Most respondents further explained that in cases where two or more plants or plant parts were combined, the synergistic or additive effects of their constituents were exploited (Table 2).

According to the responses, some of these medicinal plants were not only used for treating malaria but also for addressing various other diseases or purposes. For example, *N. latifolia* was used for diabetes, pile, back pain, and measles. *L. inermis* was used for hypertension, hernia, and measles, and used as cosmetics. *M. lucida* was used

for jaundice, hernia, and yellow fever. *C. odorata* was used for skin diseases and wounds. *T. diversifolia* was used for managing typhoid.

3.2 Antimalarial activity of medicinal plants

3.2.1 Curative test

Parasitemia was detected in the blood on day 2 post-infection, and subsequent monitoring revealed an increase in parasitemia until day 9 post-infection in both the experimental groups and negative controls. Out of the 42

Table 2. Medicinal plant used in combinations in the treatment of malaria in Omu-Aran, Kwara State

No	Plants in combinations
1	<i>Citrus aurantifolia</i> (leaves and root bark), <i>Lawsonia inermis</i>
2	<i>Magnifera indica</i> (stem bark), <i>Chromolaena odorata</i>
3	<i>Chromolaena odorata</i> , <i>Senna siamea</i> , <i>Tithonia diversifolia</i>
4	<i>Azadirachta indica</i> , <i>Carica papaya</i> , <i>Chromolaena odorata</i>
5	<i>Azadirachta indica</i> , <i>Carica papaya</i> , <i>Nauclea latifolia</i>
6	<i>Tithonia diversifolia</i> , <i>Ocimum basilicum</i> , <i>Chromolaena odorata</i>
7	<i>Magnifera indica</i> , <i>Psidium guajava</i> , <i>Chromolaena odorata</i>
8	<i>Magnifera indica</i> (male), <i>Carica papaya</i> (withered leaves), <i>Chromolaena odorata</i>
9	<i>Magnifera indica</i> , <i>Anarcadium occidentale</i> , <i>Chromolaena odorata</i>
10	<i>Magnifera indica</i> , <i>Anarcadium occidentale</i> , <i>Lawsonia inermis</i>
11	<i>Lawsonia inermis</i> , <i>Magnifera indica</i> , <i>Psidium guajava</i>
12	<i>Anarcadium occidentale</i> , <i>Azadirachta indica</i> , <i>Carica papaya</i> , <i>Chromolaena odorata</i>
13	<i>Magnifera indica</i> , <i>Anarcadium occidentale</i> , <i>Azadirachta indica</i> , <i>Chromolaena odorata</i>
14	<i>Magnifera indica</i> , <i>Anarcadium occidentale</i> , <i>Azadirachta indica</i> , <i>Morinda lucida</i>
15	<i>Magnifera indica</i> , <i>Anarcadium occidentale</i> , <i>Citrus aurantifolia</i> , <i>Lawsonia inermis</i>

doses administered in the curative tests, one dose exhibited high activity (PCS >60%), 6 showed moderate activity, 22 displayed weak activity, and 13 showed no activity (PCS ≤0) (Figure 2). The dichloromethane-methanol (DCM-MeOH) (1:1) extracts generally displayed greater antiplasmodial potential compared to the aqueous extracts. Two of the aqueous groups exhibited moderate activity, 12 had weak activity, and 13 showed no activity, while 1 DCM-MeOH (1:1) dose displayed high activity, 4 showed moderate activity, 10 exhibited weak activity, and 6 had no activity (PCS ≤0). However, despite the observed activity, there was no significant ($P < 0.05$) increase in survival days. Notably, most of the chloroquine-treated (positive) groups exhibited significant survival days (Tables 3-7). The activities elicited by selected medicinal plants are further elaborated below.

3.2.1.1 *M. lucida*

The curative activity of *M. lucida* leaves extracts ranged from inactivity (PCS ≤0%) to weak activity (PCS <30%), while the stem bark aqueous and DCM-MeOH (1:1) extracts demonstrated moderate activity with PC of 43.45% and 51.14%, respectively, at doses of 100 mg/kg and 400 mg/kg (Table 3). In terms of survival days, the highest recorded value among *M. lucida*-treated infected mice was 10.60 ± 1.95 , while positive and negative control groups had values of 18.20 ± 4.82 and 11.20 ± 2.75 , respectively.

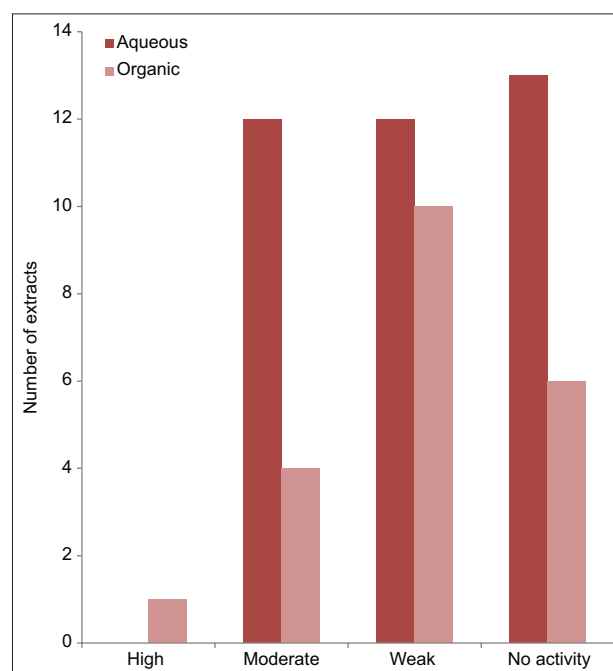


Figure 2. The curative antimalarial activity of the selected medicinal plant (aqueous and organic extracts).

3.2.1.2 *N. latifolia*

The curative antimalarial activity of the aqueous and DCM-MeOH (1:1) extracts from both the leaves and stem bark of *N. latifolia* was found to be very poor, ranging from weakly active (16.51%) to inactive (–56.93%) (Table 4). The mean survival time (MST) of *P. berghei*-infected mice treated with *N. latifolia* ranged from 6.80 ± 1.09 to 10.20 ± 1.10 days, while the groups treated with chloroquine and the vehicle had MTS between 13.00 ± 4.55 and 13.75 ± 1.89 days and 7.60 ± 0.55 to 9.80 ± 1.64 days, respectively (Table 4).

3.2.1.3 *T. diversifolia*

The organic (DCM-MeOH [1:1]) extract of *T. diversifolia* leaves exhibited high activity against established infection, with PCS of 66.13%. The aqueous extract also displayed moderate activity at the highest tested dose. However, in the 400 mg/kg organic group, most of the mice died before the end of the experiment, making it impossible to determine the PCS at this concentration (Table 5). The MST in the *T. diversifolia*-treated group ranged from 7.25 ± 0.5 to 13.00 ± 4.24 days. The positive and negative control groups had MST between 14.00 ± 2.45 to 14.67 ± 1.15 days and 7.40 ± 0.55 to 9.50 ± 1.73 days, respectively (Table 5).

3.2.1.4 *L. inermis*

L. inermis exhibited curative activity ranging from weak to moderate. The DCM-MeOH (1:1) extract at 400 mg/kg showed a PCS of 34.79% (Table 6). The activity was

Table 3. *In vivo* curative antimalarial activity of the aqueous and organic extracts of *Morinda lucida* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	Parasite density (%)	Chemosuppression (%)	Mean survival time (days)
Leaves	H ₂ O	100	76.70±3.37	-11.45	10.20±1.64
		250	55.98±0.41	18.66	9.40±1.52
		400	77.89±2.00	-13.17	10.60±1.95
Positive control	Chloroquine	5	2.01±1.75*	97.08	18.20±4.82*
Negative control	Distilled H ₂ O	0.2 mL	68.83±3.68	0.00	11.20±2.75
Leaves	Organic	100	81.93±1.45	-7.70	9.0±1.87
		250	74.08±2.24	2.61	8.60±1.34
		400	65.13±1.5	14.38	9.75±4.19
Positive control	Chloroquine	5	6.24±0.66*	91.80	14.8±2.59*
Negative control	Tween 80	0.2L	76.07±0	0.00	9.8±2.68
Stem bark	H ₂ O	100	49.67±2.54*	43.45	9.40±2.19
		250	80.86±0.46	7.93	8.00±0.00
		400	68.79±22.75	21.67	9.00±1.87
Positive control	Chloroquine	5	0.96±1.17*	98.91	14.20±4.09*
Negative control	Distilled H ₂ O	0.2 mL	87.82±8.67	0.00	8.20±1.64
Stem bark	Organic	100	N/A	17.79	7.20±1.10
		250	81.35±2.77	0.55	8.20±1.64
		400	28.88±7.51*	51.14	8.00±0
Positive control	Chloroquine	5	1.06±0.80*	98.71	11.80±1.30*
Negative control	Tween 80	0.2 mL	81.79±13.42	0.00	7.80±0.45

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

found to be dose-dependent. Among the *L. inermis*-treated groups, the highest MST recorded was 10.50 ± 2.3 days, while the positive and negative control groups had MST of 14.80 ± 2.95 and 9.00 ± 0.00 days, respectively (Table 6).

3.2.1.5 *C. odorata*

C. odorata exhibited activity ranging from weak and moderate. In the 400 mg/kg group of the aqueous extract, most of the mice died before the end of the experiment (Table 7). The MST in the *C. odorata*-treated *P. berghei*-infected mice ranged from 8.00 ± 0 to 10.50 ± 1.75 days, while the positive and negative control groups had MST between 14.67 ± 1.15 to 14.80 ± 2.95 days and 8.00 ± 0.0 to 8.67 ± 0.58 days, respectively (Table 7).

3.2.2 Repository test results

The residual antimalarial effects of the extracts were assessed on day 7 (D_7) and 9 (D_9) of the experiments. On D_7 , out of the 42 experimental groups (21 aqueous and 21 DCM-MeOH [1:1]), 3 groups exhibited high activity ($PCS > 60\%$), 19 showed moderate activity, 17 groups had weak activity, and 3 were inactive. However, on D_9 , the activities decreased, with 16 groups displaying moderate

activity, 21 showing weak activity, and 5 being inactive (Tables 8-12). Although the survival time in most of the experimental groups was higher than that of the negative controls, the differences were not statistically significant ($P < 0.05$) (Figure 3). In contrast to the suppressive and curative tests, chloroquine did not demonstrate a high percentage chemoprophylaxis (PCP). Its activity ranged from weak to inactive on D_8 , which was reflected in the survival days of the group. Aqueous extracts exhibited better antimalarial prophylactic properties, with 2 out of the 21 doses showing high activity, 12 being moderate, and 7 displaying weak activity. On the other hand, the DCM-MeOH (1:1) extracts showed a dose with high activity, 7 with moderate activity, 10 with weak activity, and 3 without activity (Figure 3). The activities elicited by selected medicinal plants are further elaborated below.

3.2.2.1 *M. lucida*

On D_7 , both the DCM-MeOH (1:1) and aqueous leaf extracts of *M. lucida* displayed activity ranging from weak ($PCP = 18\%$) to moderate ($PCP = 41.05\%$). While the activity of the DCM-MeOH (1:1) extract groups decreased on D_9 , the activity of the aqueous extract groups increased,

Table 4. *In vivo* curative antimalarial activity of the aqueous and organic extracts of *Nauclea latifolia* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	Parasite density (%)	Chemosuppression (%)	Mean survival time (days)
Leaves	H ₂ O	100	64.16±5.75	0.43	7.60±0.55
		250	64.02±11.25	0.66	9.60±1.95
		400	53.80±6.57	16.51	9.80±1.10
Positive control	Chloroquine	5	4.19±1.87*	93.50	13.00±4.55*
Negative control	Distilled H ₂ O	0.2 mL	64.44±7.10	0.00	8.80±2.05
Leaves	Organic	100	52.49±7.74	6.17	7.00±0
		250	67.13±6.69	−20.02	7.60±0.55
		400	54.73±8.73	2.15	6.80±1.09
Positive control	Chloroquine	5	4.54±2.09*	91.89	13.60±0.89*
Negative control	Tween 80	0.2 mL	55.9±36.62	0.00	7.60±0.55
Stem bark	H ₂ O	100	64.66±5.65	−5.43	9.20±1.64
		250	57.28±7.39	6.61	8.80±2.05
		400	62.34±2.43	−1.63	10.20±1.10
Positive control	Chloroquine	5	5.77±1.64*	90.59	15.00±3.32*
Negative control	Distilled H ₂ O	0.2 mL	61.33±6.27	0.00	9.80±1.64
Stem bark	Organic	100	35.50±7.87	1.66	10.0±1.87
		250	56.65±7.95	−56.93	9.0±1.87
		400	51.97±8.81	−43.95	10.00±2.45
Positive control	Chloroquine	5	5.12±4.73*	85.83	13.75±1.89*
Negative control	Tween 80	0.2 mL	36.10±16.83	0.00	8.80±2.05

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

Table 5. *In vivo* curative antimalarial activity of the aqueous and organic extracts of *Tithonia diversifolia* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	Parasite density (%)	Chemosuppression (%)	Mean survival time (days)
Leaves	H ₂ O	100	52.39±3.67	23.44	10.25±2.06
		250	57.80±0.74	15.53	10.50±1.73
		400	39.06±5.60*	42.91	13.00±4.24
Positive control	Chloroquine	5	2.65±1.24*	96.12	14.67±1.15
Negative control	Distilled H ₂ O	0.2 mL	68.43±12.14	0.00	9.50±1.73
Leaves	Organic	100	28.41±7.84*	66.13	7.33±0.58
		250	68.80±13.89	17.98	8.25±1.89
		400	N/A	N/A	7.25±0.5
Positive control	Chloroquine	5	3.03±2.58*	96.39	14.00±2.45*
Negative control	Tween 80	0.2 mL	83.88±0.52	0.00	7.40±0.55

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

although the differences were not statistically significant ($P > 0.05$) (Table 8). Unlike the DCM-MeOH (1:1) extract groups, which showed no activity at any dose levels, the aqueous extract of *M. lucida* stem bark exhibited moderate activity at all dose levels on D₆. However, the activity

decreased on D8. The MST of the *M. lucida*-treated mice in this experiment ranged from 10.00 ± 0.82 to 12.50 ± 1.73 days, when the positive and negative control groups had MST between 10.75 ± 0.96 to 12.50 ± 2.38 days and 10.50 ± 1.00 to 11.25 ± 1.89 days, respectively (Table 8).

Table 6. *In vivo* curative antimalarial activity of the aqueous and organic extracts of *Lawsonia inermis* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	Parasite density (%)	Chemosuppression (%)	Mean survival time (days)
Leaves	H ₂ O	100	61.24±5.14	3.45	10.50±1.73
		250	49.64±6.29	21.73	10.50±2.3
		400	N/A	9.07	9.0±0
Positive control	Chloroquine	5	2.65±1.24*	95.82	14.67±1.15*
Negative control	Distilled H ₂ O	0.2 mL	63.43±2.87	0.00	9.00±0
Leaves	Organic	100	64.38±7.17	14.81	8.60±1.34
		250	66.35±4.18	12.19	7.80±0.45
		400	49.28±3.77*	34.79	8.33±0.58
Positive control	Chloroquine	5	1.24±0.70*	98.36	14.80±2.95*
Negative control	Tween 80	0.2 mL	75.56±5.23	0.00	8.00±0

Notes: *Indicates significant difference from control, $P \leq 0.05$; chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

Table 7. *In vivo* curative antimalarial activity of the aqueous and organic extracts of *Chromolaena odorata* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	Parasite density (%)	Chemosuppression (%)	Mean survival time (days)
Leaves	H ₂ O	100	58.19±1.08	14.96	10.50±1.73
		250	54.04±5.70	21.02	8.00±0.00
		400	N/A	N/A	8.33±0.58
Positive control	Chloroquine	5	2.65±1.24*	96.12	14.67±1.15*
Negative control	Distilled H ₂ O	0.2 mL	63.43±2.87	0.00	8.67±0.58
Leaves	Organic	100	67.14±8.00	11.15	8.00±0.00
		250	50.42±8.38*	33.28	10.20±3.49
		400	43.83±2.78*	42	10.20±4.66
Positive control	Chloroquine	5	1.24±0.70*	98.36	14.80±2.95*
Negative control	Tween 80	0.2 mL	75.57±3.70	0.00	8.00±0

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

3.2.2.2 *N. latifolia*

On day 7 of the experiment, when blood smears were examined and percentage parasitemia was determined, both the DCM-MeOH (1:1) and aqueous leaf extracts of *N. latifolia* exhibited moderate antiplasmodial activity (Table 9). However, this activity decreased on D₈. In contrast, the DCM-MeOH (1:1) and aqueous stem bark extract of *N. latifolia* demonstrated high activity, with PCP ranging from 61.51% to 81.69%, which then decreased to moderate activity by day 9. The mean survival days in *N. latifolia*-treated groups ranged from 10.25 ± 0.05 to 13.25 ± 2.22, with the highest MST observed in the aqueous stem bark extract group at 250 mg/kg. The highest MST in the positive and negative control groups were 12.50 ± 2.38 days and 11.25 ± 1.89 days, respectively (Table 9).

3.2.2.3 *T. diversifolia*

On D₇, the repository activity of *T. diversifolia* ranged from very weak (9.3%) to moderate (54.59%). However, by D₉, the activity observed in most of the groups had decreased (Table 10). The MST of the *T. diversifolia*-treated mice ranged from 10.25 ± 0.50 to 15.00 ± 3.08 days, while the highest MST observed in the chloroquine and vehicle-treated mice were 12.00 ± 4.69 days and 12.20 ± 1.79 days, respectively (Table 10).

3.2.2.4 *L. inermis*

The assessment of the *in vivo* antiplasmodial prophylactic ability of *L. inermis* revealed a range of very weak (4.65%) to moderate (34.78%) activity on D₆. However, on D₉, the results for PCP were inconsistent, as some groups showed a decrease in activity while others displayed an

Table 8. *In vivo* repository antimalarial activity of the aqueous and organic extracts of *Morinda lucida* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	D ₆ Parasite density (%)	D ₆ Chemoprophylaxis (%)	D ₈ Parasite density (%)	D ₈ Chemoprophylaxis (%)	Mean survival time (days)
Leaves	H ₂ O	100	7.18±1.09*	35.33	31.70±4.68*	39.96	11.25±1.89
		250	8.97±3.14	19.12	41.05±0.86	22.27	10.75±0.5
		400	6.54±0.48*	41.05	27.50±4.95*	47.92	12.50±1.73
Positive control	Chloroquine	5	8.76±2.47	21.02	52.98±3.51	-0.32	12.25±2.06
Negative control	Distilled H ₂ O	0.2 mL	11.10±4.99	0.00	52.81±3.53	0.00	10.75±0.5
Leaves	Organic	100	7.90±2.70	18.46	53.84±4.45	9.87	11.0±1.73
		250	7.57±1.57	21.9	52.73±2.74	11.72	11.40±3.21
		400	7.05±1.69	27.26	48.21±5.25	19.3	11.20±1.64
Positive control	Chloroquine	5	7.34±4.62	24.25	67.48±3.28	-12.97	10.80±0.45
Negative control	Tween 80	0.2 mL	9.69±3.28	0.00	59.73±3.14	0.00	11.25±1.89
Stem bark	H ₂ O	100	3.84±0.69*	39.36	38.15±2.88	20.19	10.0±0.82
		250	3.47±1.30*	45.26	37.56±1.15	21.42	10.33±0.58
		400	2.82±0.72*	55.52	32.48±2.97*	32.06	10.75±0.96
Positive control	Chloroquine	5	4.40±1.05*	30.52	42.77±4.56	10.53	10.75±0.96
Negative control	Distilled H ₂ O	0.2 mL	6.33±1.30	0.00	47.80±2.42	0.00	10.50±1.00
Stem bark	Organic	100	13.45±3.51	-18.12	60.07±6.30	-0.88	11.75±2.87
		250	12.09±6.71	-6.1	66.60±2.57	-11.86	10.25±0.5
		400	13.49±2.49	-18.44	82.85±1.74	-39.14	10.33±0.58
Positive control	Chloroquine	5	9.85±1.89	13.56	58.34±4.71	2.02	12.50±2.38
Negative control	Tween 80	0.2 mL	11.39±0.75	0.00	59.54±3.56	0.00	11.25±1.89

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; $30 - 60\%$, moderate activity; $<30\%$, weak activity; 0% , inactivity^[13].

insignificant increase ($P > 0.05$) (Table 11). The MST in *L. inermis*-treated group ranged from 10.50 ± 0.58 to 12.00 ± 1.83 days, while the positive and negative control groups had MST ranging from 10.75 ± 0.96 to 12.20 ± 1.64 days and 10.50 ± 1.00 to 11.60 ± 0.55 days, respectively.

3.2.2.5 *C. odorata*

On D₇, the extracts of *C. odorata* extracts exhibited prophylactic activity ranging from weak (13.57%) to moderate (56.21%), with the DCM-MeOH (1:1) extract groups showing better activity (Table 12). The MST ranged from 10.00 ± 0.82 to 12.60 ± 2.19 days for the mice treated with *C. odorata* extracts. The positive and negative control groups had MST ranging from 10.75 ± 0.96 to 12.20 ± 1.64 days and 10.50 ± 1.00 to 11.60 ± 0.55 days, respectively (Table 12).

3.2.3 Comparisons of curative and repository test results

Generally, better *in vivo* antiplasmodial activity was observed when extracts were administered before infection (Repository test) than after the establishment of infection

(curative test). This is observed in all the extracts tested in Figure 4.

4 Discussion

The ethnobotanical survey conducted in Omu-Aran showed that a variety of medicinal plants are traditionally used in the community for treating malaria. These plants are mostly consumed orally in the form of decoction. Thirty-one species from 24 plant families were documented during the survey conducted in this study, indicating the relevance of the medicinal flora in the day-to-day management of malaria in the area. Furthermore, most of the mentioned medicinal plants have been reported in other parts of Nigeria and Africa, and studies have investigated their efficacy, yielding positive results. This confirms the validity of the gathered information^[25,26].

In the traditional medicine system, certain herbal medicines are usually used for prevention before the onset of disease, while others are used after an infection has already occurred. It is noteworthy that the same plants often serve both preventive and curative purposes. Therefore, this study aimed to evaluate the antimalarial

Table 9. *In vivo* repository antimalarial activity of the aqueous and organic extracts of *Nauclea latifolia* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	D ₆ Parasite density (%)	D ₆ chemoprophylaxis (%)	D ₈ parasite density (%)	D ₈ chemoprophylaxis (%)	Mean survival time (days)
Leaves	H ₂ O	100	5.02±1.34*	54.78	28.84±1.83*	45.39	12.25±2.06
		250	6.00±1.14*	45.92	29.94±4.26*	43.3	12.25±2.06
		400	9.44±3.45	14.92	48.60±1.94	7.97	11.50±1.73
Positive control	Chloroquine	5	8.76±2.47	21.02	52.98±3.51	-0.32	12.25±2.06
Negative control	Distilled H ₂ O	0.2 mL	11.10±4.99	0.00	52.81±2.54	0.00	10.75±0.5
Leaves	Organic	100	7.63±2.05*	33.04	47.78±3.50	27.22	11.50±1.73
		250	4.89±3.98*	57.1	35.20±3.02*	46.38	10.25±0.5
		400	7.60±4.40*	33.3	48.44±4.90	26.21	12.75±2.75
Positive control	Chloroquine	5	9.85±1.89	13.56	63.64±1.74	3.06	12.50±2.38
Negative control	Tween 80	0.2 mL	11.39±0.75	0.00	66.65±3.57	0.00	11.25±1.89
Stem bark	H ₂ O	100	8.72±3.72	21.23	42.56±1.84	19.41	11.67±2.08
		250	4.27±2.27*	61.51	26.32±0.56*	50.16	13.25±2.22
		400	4.01±1.52*	63.9	26.22±2.74*	50.35	12.67±2.31
Positive control	Chloroquine	5	8.76±2.47	21.02	49.16±1.48	6.9	11.67±2.08
Negative control	Distilled H ₂ O	0.2 mL	11.10±4.99	0.00	52.81±1.56	0.00	10.75±0.5
Stem bark	Organic	100	7.32±2.85	24.5	58.90±2.41	2.21	10.40±0.55
		250	7.64±2.87	21.13	48.61±2.89	19.30	11.00±1.73
		400	1.78±1.90*	81.69	30.40±2.56*	49.53	11.40±1.67
Positive control	Chloroquine	5	7.34±4.62	24.25	67.48±3.28	-12.02	10.50±0.45
Negative control	Tween 80	0.2 mL	9.69±3.28	0.00	60.23±3.93	0.00	11.25±1.89

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

activity of *N. latifolia*, *L. inermis*, *T. diversifolia*, *C. odorata*, and *M. lucida*. The evaluation was conducted both before the establishment of infection and after using *P. berghei*, which causes a disease similar to human plasmodium infections^[27].

The antiplasmodial activities varied depending on the type of plant, plant part, method of extraction, and parasite. Earlier researchers have reported the antimalarial activities of these plants, although they were tested using different methods^[13,28-36]. However, there was a difference in the *in vivo* antiplasmodial activity of the extracts, with aqueous extracts appearing to be more effective than organic extracts.

In this study, the efficacy of the extracts was measured based on the percentage of chemo suppressions/

chemoprophylaxis against parasitemia in mice and the MST of the animals. The *in vivo* antimalarial activity of the extracts exhibited a significant difference in parasite density reduction and survival days ($P < 0.05$) between the extract-treated groups and the group treated with the vehicle (negative control). The observed reduction in parasitemia in the treated groups, compared to the negative control groups, indicates the antimalarial potential of the extracts.

In this study, it was observed that the extracts *M. lucida* generally exhibited between moderate and weak activity in the tests, despite being reported as one of the five most frequently used antimalarial herbs in folk medicine, especially in South-west Nigeria^[36,37]. The moderate activity of *M. lucida* against *P. falciparum* was also observed in

Table 10. *In vivo* repository antimalarial activity of the aqueous and organic extracts of *Tithonia diversifolia* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	D ₆ Parasite density (%)	D ₆ Chemoprophylaxis (%)	D ₈ Parasite density (%)	D ₈ Chemoprophylaxis (%)	Mean survival time (%)
Leaves	H ₂ O	100	3.81±0.61*	30.65	34.22±2.71	10.96	14.4±1.67
		250	2.50±1.16*	54.59	25.12±4.38*	34.64	12.2±4.09
		400	3.31±3.00*	39.73	26.88±0.99*	30.06	15.0±3.08
Positive control	Chloroquine	5	3.32±0.63*	39.61	41.40±5.45	-7.74	12.0±4.69
Negative control	Distilled H ₂ O	0.2 mL	5.50±1.48	0.00	38.43±3.18	0.00	12.2±1.79
Leaves	Organic	100	4.45±2.25*	33.78	36.0±2.19*	32.97	13.75±3.86
		250	5.75±1.39	14.51	48.47±4.80	9.76	10.25±0.50
		400	6.10±0.67	9.3	39.76±3.44	25.97	12.50±1.73
Positive control	Chloroquine	5	6.46±2.85	3.92	46.84±4.98	12.79	10.75±0.50
Negative control	Tween 80	0.2 mL	6.72±1.47	0.00	53.71±1.92	0.00	10.75±0.50

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

Table 11. *In vivo* repository antimalarial activity of the aqueous and organic extracts of *Lawsonia inermis* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	D ₆ parasite density (%)	D ₆ Chemoprophylaxis (%)	D ₈ Parasite density (%)	D ₈ Chemoprophylaxis (%)	Mean survival time (%)
Leaves	H ₂ O	100	5.36±1.79	15.41	38.33±1.58	19.81	12.00±1.83
		250	5.47±0.98	13.57	47.40±2.40	0.85	10.50±0.58
		400	4.13±1.27*	34.78	35.18±7.64	26.41	10.50±1.73
Positive control	Chloroquine	5	4.40±1.05*	30.52	42.77±4.56	10.53	10.75±0.96
Negative control	Distilled H ₂ O	0.2 mL	6.33±1.30	0.00	47.80±2.42	0.00	10.50±1.00
Leaves	Organic	100	8.11±1.71	4.65	38.29±1.84	15.68	12.00±1.73
		250	5.84±2.57*	31.35	26.99±2.63*	40.57	11.80±1.79
		400	7.86±1.69	27.26	29.97±0.68*	34.01	11.60±1.95
Positive control	Chloroquine	5	5.87±1.59*	30.92	38.53±5.11	15.15	12.20±1.64
Negative control	Tween 80	0.2 mL	8.50±1.00	0.00	45.41±3.07	0.00	11.60±0.55

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

the unpublished work by Makinde and colleagues, cited in^[38]. This study demonstrated an interplay between host immunity and the activity of *M. lucida*. In adults, *M. lucida* (leaves extract) showed 100% parasite clearance, whereas no such clearance was observed in children, who have a less well-developed immune response. Meanwhile, children are the group at greatest risk of mortality, making efficacy particularly important in this population.

The leaves and stem bark of *N. latifolia* demonstrated relatively good activity in this study, particularly in the repository test. The aqueous stem bark extract exhibited high activity at 250 and 400 mg/kg, while the organic extract showed high activity at 400 mg/kg. Earlier studies conducting preliminary phytochemical screening of the leaf and stem bark extracts of this plant reported the presence of bioactive compounds such as alkaloids,

Table 12. *In vivo* repository antimalarial screening of the aqueous and organic extracts of *Chromolaena odorata* in *Plasmodium berghei*-infected mice

Plant part	Extract	Dose (mg/kg)	D ₆ Parasite density (%)	D ₆ Chemoprophylaxis (%)	D ₈ Parasite density (%)	D ₈ Chemoprophylaxis (%)	Mean survival time (%)
Leaves	H ₂ O	100	2.78±2.67	15.41	36.52±2.76	23.61	11.75±0.96
		250	5.47±0.98	13.57	40.06±2.40	16.19	10.50±0.58
		400	4.13±1.27*	34.78	49.40±7.64	−3.34	10.00±0.82
Positive control	Chloroquine	5	4.40±1.05*	30.52	42.77±4.56	10.53	10.75±0.96
Negative control	Distilled H ₂ O	0.2 mL	6.33±1.30	0.00	47.80±2.42	0.00	10.50±1.00
Leaves	Organic	100	7.23±1.26	14.91	29.59±4.72	15.68	12.60±2.19
		250	4.21±2.12*	50.47	28.31±3.86*	40.57	12.20±1.64
		400	3.72±2.03*	56.21	36.17±1.40*	34.01	12.20±1.64
Positive control	Chloroquine	5	5.87±1.59*	30.92	38.53±5.11	15.15	12.20±1.64
Negative control	Tween 80	0.2 mL	8.50±1.00	0.00	45.41±3.07	0.00	11.60±0.55

Notes: *Indicates significant difference from control, $P \leq 0.05$. Chemosuppression: $\geq 60\%$, high activity; 30 – 60%, moderate activity; $< 30\%$, weak activity; 0%, inactivity^[13].

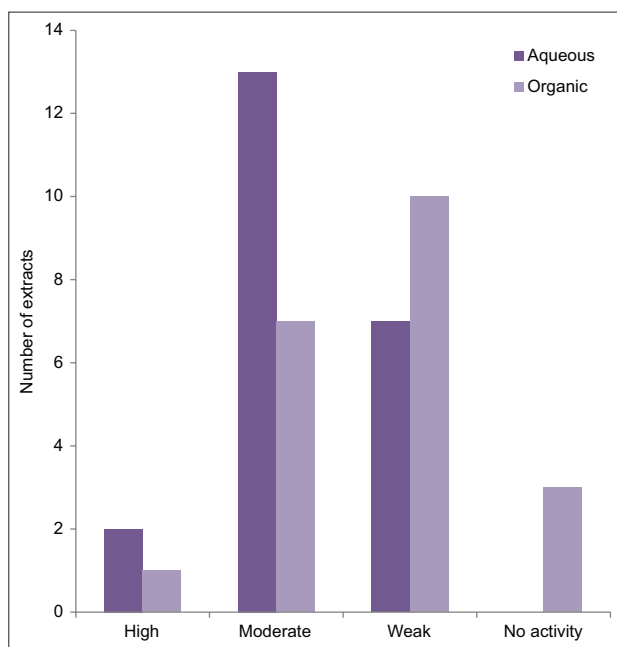


Figure 3. *In vivo* antiplasmodial activities of the selected medicinal plant extracts in repository and curative tests.

saponins, flavonoids, tannins, and terpenes^[39-41]. These phytochemical compounds increase the level of oxidative molecules in the cells, thereby inhibiting protein synthesis in the parasites^[42]. This mechanism could nullify the oxidative damage induced by the malarial parasite. It also suggests that the antiplasmodial activity of the ethanolic leaf extract of *N. latifolia* may be attributed to the antioxidant and

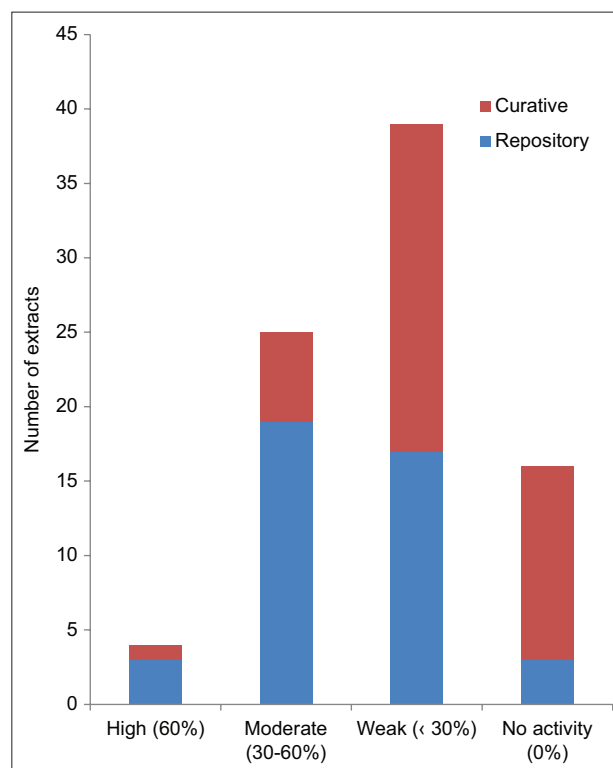


Figure 4. *In vivo* antiplasmodial activities of the selected medicinal plant extracts in repository and curative tests.

antiplasmodial effects of these phytochemicals^[43]. These findings corroborate the reported antiplasmodial activity of the plant and support further evidence for its traditional

use. Furthermore, other studies^[31,35] have demonstrated good *in vivo* antiplasmodial activity of *N. latifolia*, with the highest activity observed in the prophylactic test.

Repository experiments revealed that chloroquine is a very weak prophylactic agent, which can be attributed to its mechanism of action as a blood schizontocidal agent. Chloroquine, like other quinine derivatives, requires the presence of free heme to act. The free heme produced during the hydrolysis of hemoglobin is toxic to the parasite and, therefore, must be removed or detoxified. However, the malaria parasite lacks enzymes for detoxification, and therefore, heme is detoxified by converting it into a nontoxic malaria pigment called hemozoin. Chloroquine inhibits the detoxification reaction by binding to heme, hence preventing the destruction of parasites in liver cells where no hemoglobin digestion takes place^[44]. Consequently, the singular use of chloroquine in the prevention and treatment of malaria has led to the widespread distribution of chloroquine-resistant strains. The discontinuation of chloroquine usage has reduced the drug pressure, resulting in a decrease in chloroquine-resistant *Plasmodium* parasites^[45]. It was observed that after 12 years of chloroquine withdrawal from use in Malawi, its efficacy in the treatment of malaria has shown signs of returning^[46]. Similar observations of chloroquine efficacy returning against *Plasmodium vivax* malaria in Ethiopia and the Saharan Zone in Mauritania after the use of artemisinin-based combination therapy have been reported^[47,48]. Moreover, chloroquine has shown a greater chemo suppressive activity than the extracts and fractions of the plants used in this study in the curative test. However, due to resistance, chloroquine is no longer used as an antimalarial agent in humans. The advantage that these plants have over chloroquine is that they are new, effective, and not associated with known resistance.

The extracts generally exhibited weak to moderate activity in the repository test, with *N. latifolia* showing high activity at certain concentrations. However, in the curative test, the majority of the extracts, especially *N. latifolia*, displayed between no to weak activity. The antiplasmodial effect of *M. lucida* on established malaria infection in mice showed significant malaria suppression compared to the other extracts. These findings suggest that *M. lucida* could be a better curative agent rather than a prophylactic agent, while other extracts, especially *N. latifolia*, could be considered prophylactic agents.

5 Conclusion

The study investigated the plants used for treating malaria in Omu Aran Kwara State, Nigeria, and revealed that the extracts of *M. lucida*, *N. latifolia*, *C. odorata*, *T. diversifolia*,

and *L. inermis* have both curative and repository effects in the mice infected with *P. berghei*. However, the antimalarial effect of the extracts was found to be higher when administered before infection rather than after infection. These results agree with the traditional use of these plants and provide a scientific basis for their continuous use. The findings in the present study encourage further research on these plants, which have demonstrated antiplasmodial activity, with the view to developing new antiplasmodial drugs.

Acknowledgments

The authors would like to thank the respondents to the questionnaires in Omu-Aran, Kwara State, Nigeria, and equally appreciate the director of the Centre for Traditional Medicine and Drug Research (CTMDR). The technical assistance of Mr. Enoch Moindi and Mr. Ogutu of KEMRI animal house during the *in vivo* antiplasmodial experiment is well appreciated.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: Funmilayo I. D. Afolayan

Investigation: Funmilayo I. D. Afolayan, Beatrice Irungu

Writing – original draft: Funmilayo I. D. Afolayan

Writing – review & editing: Olayemi Adegbolagun, Jennifer Orwa, Chiaka Anumudu

All authors read and approved the final manuscript.

Ethics approval and consent to participate

The respondents to the ethnobotanical survey questionnaires verbally consented to participate in the survey. The guidelines provided by the WHO and the ACUC of KEMRI were adhered to, which outline the internationally recognized principles for the use and care of laboratory animals.

Consent for publication

Not applicable.

Availability of data

Supporting data can be obtained from the corresponding author following formal request.

References

- World Health Organization. World Malaria Day 2022. Geneva: World Health Organization. 2022. Available from: <https://www.who.int/campaigns/world-malaria-day/2022> [Last accessed on 2022 Jun 06].
- Osakede UA, Lawanson AO, Sobowale DA, 2017, Entrepreneurial interest and academic performance in Nigeria: Evidence from undergraduate students in the university of Ibadan. *J Innov Entrep*, 6: 19.
<https://doi.org/10.1186/s13731-017-0079-7>
- Tadesse SA, Wubneh ZB, 2017, Antimalarial activity of *Syzygium guineense* during early and established *Plasmodium* infection in rodent models. *BMC Complement Altern Med*, 17: 21.
<https://doi.org/10.1186/s12906-016-1538-6>
- Rai SK, Apoorva Rai, KK, Pandey-Rai S, 2021, Chapter 15-New perspectives of the *Artemisia* annual bioactive compounds as an affordable cure in the treatment of malaria and cancer. In: Sinha RP, Donat H, editors. *Natural Bioactive Compounds*. Cambridge: Academic Press, p299–315.
- Vijayaraghavan K, Rajkumar J, Bukhari SN, *et al.*, 2017, *Chromolaena odorata*: A neglected weed with a wide spectrum of pharmacological activities (review). *Mol Med Rep*, 15: 1007–1016.
<https://doi.org/10.3892/mmr.2017.6133>
- Azmi SN, Mohamad CW, Kasim KE, 2019, Encapsulation of *C. odorata* extracts for antimicrobial activity. *J Phys Conf Ser*, 1372: 012046.
<https://doi.org/10.1088/1742-6596/1372/1/012046>
- Dalziel JK, 1957, *The Useful Plants of West Tropical Africa*. 2nd ed. London: Crown Agents, p361.
- Kokwaro JO, 1976, *Medicinal Plants of East Africa*. Nairobi: East Africa Literature Bureau, p35.
- Alyazji AA, Gheneima NA, 2011, Antibacterial, antifungal and synergistic effect of *Lawsonia inermis*, *Punica granatum* and *Hibiscus sabdariffa*. *Ann Alquds Med*, 7: 33–41.
- Zumrutdal E, Ozaslan MA, 2012, Miracle plant for the herbal pharmacy; Henna (*Lawsonia inermis*). *Int J Pharmacol*, 8: 483–489.
- WFO, 2022, The World Flora Online. Available from: <https://www.worldfloraonline.org/search?query=lawsonia+inermis> [Last accessed on 2022 Jun 25].
- Hema R, Kumaravel S, Gomathi N, 2010, Gas chromatography mass spectroscopic analysis of *Lawsonia inermis* leaves. *N Y Sci J*, 3(12): 99–101.
- Afolayan FI, Adegbolagun OM, Irungu B, *et al.*, 2016, Antimalarial Actions of *Lawsonia inermis*, *Tithonia diversifolia* and *Chromolaena odorata* in combination. *J Ethnopharmacol*, 191: 188–194.
<https://doi.org/10.1016/j.jep.2016.06.045>
- Afolayan FI, Sulaiman KA, Okunade WT, 2020, Ethnobotanical survey of plants used in cancer therapy in Iwo and Ibadan, South-Western of Nigeria. *J Pharm Pharmacogn Res*, 8(5): 346–367.
- Adeneye AA, Agbaje EO, 2008, Pharmacological evaluation of oral hypoglycemic and antidiabetic effects of fresh leaves ethanol extract of *Morinda lucida* benth. In normal and alloxan-induced diabetic rats. *Afr J Biomed Res*, 11: 65–71.
<https://doi.org/10.4314/ajbr.v11i1.50669>
- Owolabi SL, Azeze IA, Boyejo AO, *et al.*, 2019, Antidermatophytic effect of *M. Lucida* part extracts (ethanolic and aqueous) on selected clinical isolates. *J Med Sci Clin Res*, 7(9): 188–194.
<https://doi.org/10.18535/jmscr/v7i9.30>
- Adeneye AA, Olagunju JA, Olatunji BH, *et al.*, 2017, Modulatory effect of *Morinda lucida* aqueous stem bark extract on blood glucose and lipid profile in alloxan-induced diabetic rats. *Afr J Biomed Res*, 20: 75–84.
- Adeleye OO, Ayeni OJ, Ajamu MA, 2018, Traditional and medicinal uses of *Morinda lucida*. *J Med Plants Stud*, 6(2): 249–254.
- Al-Snafi AE, 2019, A review on *Lawsonia inermis*: A potential medicinal plant. *Int J Curr Pharm Res*, 11(5): 1–13.
<https://doi.org/10.22159/ijcpr.2019v11i5.35695>
- Peters W, 1965, Drug resistance in *Plasmodium berghei* vincke and lips. 1948. I. Chloroquine resistance. *Exp Parasitol*, 17: 80–89.
[https://doi.org/10.1016/0014-4894\(65\)90012-3](https://doi.org/10.1016/0014-4894(65)90012-3)
- Ryley JF, Peters W, 1970, The antimalarial activity of some quinolone esters. *Ann Trop Med Parasitol*, 64: 209–222.
<https://doi.org/10.1080/00034983.1970.11686683>
- Elufioye TO, Agbedahunsi JM, 2004, Antimalarial activities of *Tithonia diversifolia* (Asteraceae) and *Crossopteryx febrifuga* (Rubiaceae) on mice *in vivo*. *J Ethnopharmacol*, 93(2–3): 167–171.
<https://doi.org/10.1016/j.jep.2004.01.009>
- Abosi AO, Raseroke BH, 2003, *In vivo* antimalarial activity of *Vernonia amygdalina*. *Br J Biomed Sci*, 60(2): 89–91.
<https://doi.org/10.1080/09674845.2003.11783680>
- Abatan MO, Makinde MJ, 1986, Screening *Azadirachta indica* and *Pisum sativum* for possible antimalarial activities. *J Ethnopharmacol*, 17: 85–93.
[https://doi.org/10.1016/0378-8741\(86\)90075-9](https://doi.org/10.1016/0378-8741(86)90075-9)
- Lagnika L, Djehoue R, Yedomonhan H, 2016, Ethnobotanical survey of medicinal plants used in malaria management in South Benin. *J Med Plants Res*, 10(41): 748–756.
<https://doi.org/10.5897/JMPR2016.6219>

26. Doatinsa SE, Dossa CP, Yedomonhan H, 2016, Ethnobotanical study of plants used by the Lacustrine population of Southern Benin in the treatment of malaria. *J Pharmacogn Phytochem*, 5(6): 430–436.
27. Mishra S, Sharma H, Mishra R, *et al.*, 2014, A review on antimalarial drug discovery and its screening method. *World J Pharm Pharm Sci*, 8: 1288–1304.
28. Bello IS, Oduola T, 2009, Evaluation of antimalarial activity of various fractions of *Morinda lucida* leaf extract and *Alstonia boonei* stem bark. *Glob J Pharmacol*, 3(3): 163–165.
29. Abdullah WO, Unyah NZ, Hamat RA, *et al.*, 2011, *In vitro* antiplasmodial activity and cytotoxicity of ten plants used as traditional medicine in Malaysia. *J Sains Kesihatan Malaysia*, 9(2): 5–8.
30. Arise RO, Malomo SO, Bolarinwa OA, 2013, Comparative antimalarial and safety attributes of methanolic extract of leaves of *Tithonia diversifolia* and *Morinda lucida* in animal models. *Am J Drug Discov Dev*, 3(3): 140–148.
<https://doi.org/10.3923/ajdd.2013.140.148>
31. Adebajo AC, Odediran SA, Aliyu FA, *et al.*, 2014, *In vivo* antiplasmodial potentials of the combinations of four Nigerian antimalarial plants. *Molecules*, 19(9): 13136–13146.
<https://doi.org/10.3390/molecules190913136>
32. Ayoade GW, Olusi TA, Amoo IA, *et al.*, 2014, Composition of some traditional malaria remedies and their antiplasmodial effects on *Plasmodium berghei*. *Int J Sci Res Publ*, 4(3): 1–8.
33. Ezenyi IC, Salawu OA, Kulkarni R, *et al.*, 2014, Antiplasmodial activity-aided isolation and identification of quercetin-4'-methyl ether in *Chromolaena odorata* leaf fraction with high activity Against chloroquine-resistant *Plasmodium falciparum*. *Parasitol Res*, 113(12): 4415–4422.
<https://doi.org/10.1007/s00436-014-4119-y>
34. Majesty D, Benjamin A, Amadike U, *et al.*, 2014, Effect of “Udu”, an antimalarial herbal preparation on visceral organ weight and blood lipid profiles in wistar rats. *J Phys Conf Ser*, 8: 1–7.
35. Ogbuehi IH, Ebong OO, Asuquo EO, *et al.*, 2014, Evaluation of the anti-plasmodial activity of the methanolic root extracts of *Anthocleista nobilis* G. Don, *Nauclea latifolia* Smith and *Napoleona imperialis* P. Beauv. *Br J Pharmacol Toxicol*, 5(2): 75–82.
36. Olasehinde GI, Ojurongbe O, Adeyeba AO, *et al.*, 2014, *In vitro* studies on the sensitivity pattern of *Plasmodium falciparum* to anti-malarial drugs and local herbal extracts. *Malar J*, 13(1): 63.
<https://doi.org/10.1186/1475-2875-13-63>
37. Lawal HO, Etatuvie SO, Fawehinmi AB, 2012, Ethnomedicinal and pharmacological properties of *Morinda lucida*. *J Nat Prod*, 5: 93–99.
38. Willcox ML, Bodeker G, Rasoanaivo P, 2004, Traditional Medicinal Plants and Malaria. Guidelines for Clinical Studies on Herbal Anti-malarials. New York: CRC Press, p321–338.
39. Bolaji O, John O, Toluani FE, *et al.*, 2018, Evaluation of phytochemical constituents and antimicrobial activity of leaves and stem bark extracts of *Sarcocephalus latifolius*. *Microbiol Res J Int*, 24(2): 1–10.
<https://doi.org/10.9734/MRJI/2018/41887>
40. Ochalefu DO, Adoga GI, Luka CD, *et al.*, 2018, Phytochemical composition and effect of *Nauclea latifolia* aqueous extracts on blood glucose levels of streptozotocin-induced diabetic wistar albino rats. *J Biomed Res Clin Pract*, 1(2): 157–163.
41. Odeniyi MA, Okumah VC, Adebayo-Tayo BC, *et al.*, 2020, Green synthesis and cream formulations of silver nanoparticles of *Nauclea latifolia* (African peach) fruit extracts and evaluation of antimicrobial and antioxidant activities. *Sustain Chem Pharm*, 15: 100197.
42. Laryea MK, Borquaye LS, 2019, Antimalarial efficacy and toxicological assessment of extracts of some Ghanaian medicinal plants. *J Parasitol Res*, 2019: 1630405.
<https://doi.org/10.1155/2019/1630405>
43. Lawal B, Shittu OK, Oibiokpa FI, *et al.*, 2017, African natural products with potential antioxidants and hepatoprotectives properties: A review. *Clin Phytosci*, 2: 23.
<https://doi.org/10.1186/s40816-016-0037-0>
44. Kumar S, Bhardwaj TR, Prasad DN, *et al.*, 2018, Drug targets for resistant malaria: Historic to future perspectives. *Biomed Pharmacother*, 104: 8–27.
<https://doi.org/10.1016/j.biopha.2018.05.009>
45. Ocan M, Akena D, Nsoby S, *et al.*, 2019, Persistence of chloroquine resistance alleles in malaria endemic countries: A systematic review of burden and risk factors. *Malar J*, 18: 76.
<https://doi.org/10.1186/s12936-019-2716-z>
46. Laufer MK, Plowe CV, 2004, Withdrawing antimalarial drugs: Impact on parasite resistance and implications for malaria treatment policies. *Drug Resist Updat*, 7: 279–288.
<https://doi.org/10.1016/j.drug.2004.08.003>
47. Mekonnen SK, Medhin G, Berhe N, *et al.*, 2014, Return of chloroquine-sensitive *Plasmodium falciparum* parasites and emergence of chloroquine-resistant *Plasmodium vivax* in Ethiopia. *Malar J*, 13(1): 244.
<https://doi.org/10.1186/1475-2875-13-244>
48. Salem MS, Lemine YO, Deida JM, *et al.*, 2015, Efficacy of chloroquine for the treatment of *Plasmodium vivax* in the Saharan zone in Mauritania. *Malar J*, 14: 39.
<https://doi.org/10.1186/s12936-015-0563-0>