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Evaluation of the Antimalarial Activity of *Garcinia kola* Ethanolic Extracts in the Prevention and Treatment of *Plasmodium berghei*-infected Mice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study aimed to evaluate of the anti-malarial activity of *Garcinia kola* (bitter kola) nut and leaf ethanolic extracts in Treatment of *Plasmodium berghei*-infected Mice. The two plant parts were collected from its natural breeding site and were processed in the laboratory, the phytochemical quantitative and qualitative analysis was conducted. The experiment was designed to access the curative and prophylactic antimalarial activity of the plant extracts. *P. berghei* was obtained from a donor mouse. The blood from the donor mouse was collected and diluted with 0.8% normal saline

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which was used to infect the test-groups used in this experiment. For prophylactic group, the mice were given extracts prior to the infection but for the curative, the mice were infected with the parasite before the administration of the plant extracts. In all cases, the plant extract administration was done by compulsory oral intubations with the aid of cannula and syringe. The curative test results for the nut extract showed dose-dependent parasitemia reduction and suppression, with the highest dose (600 mg/kg) exhibiting 61.82% suppression and a mean survival time (MST) of 17.20±0.23 days. Similarly, the leaf extract at 600 mg/kg demonstrated 39.94% suppression and an MST of 16.28±0.37 days. In the prophylactic test, the nut and leaf extracts exhibited significant parasitemia suppression, with the nut extract at 200 mg/kg achieving 92.36% suppression and an MST of 30.00±0.00 days. These findings suggest that both *Garcinia kola* nut and leaf ethanolic extracts contain bioactive compounds with notable antimalarial properties.

Keywords: Antimalarial activity; curative efficacy; ethanolic extracts; garcinia kola; phytochemical analysis; prophylactic efficacy.

1. INTRODUCTION

"Malaria remains one of the most serious world health problems and the major cause of morbidity and mortality in endemic areas. Antimalarial drugs are expensives that majority of Nigerians especially those residing in rural areas cannot afford to buy. Individuals that can afford it are not equally safe due to the increasing prevalence of drug-resistant strains of *Plasmodium* species to the frontline antimalarial drugs. In addition, management of malaria is a big challenge because currently available indoor spraying with insecticides to reduce the transmission of malaria is hampered by insecticide resistance; absence of clinically proven vaccine; lack of the necessary infrastructure and resources to manage and control malaria as well as fake drugs. Indeed, artemisinin resistance to a deadly *Plasmodium falciparum* now poses a threat to the control and elimination of malaria" (Ashley et al., 2014). Chloroquine resistant strains can be controlled by artemisinin and artemisinin derivatives. But, currently there is no alternative approved antimalarial drug to replace artemisinin derivatives.

In spite of promising progress in controlling the diseases, malaria remains one of the major public health problems in the Africa in that it has a heavy burden on individual families and national health systems. In many African countries 30 % or more of outpatient's visit and hospital admissions are due to malaria (President's Malaria Initiative [PMI], 2014). "Malaria affects the economy in which it follows different channels. The direct effect is that adults are unable to work during episodes of the disease, and may significantly weaken an individual for a period afterward. Malaria also retards economic and social development through effects such as reduced working hours due to sickness or attending to the sick, income spent on financing health care, which in turn, lead to impacts at national level due to massive health care budgets, reduced productivity of the work force, and the like. The disease subsists in more than 100 countries in different regions of the world, including India, Southeast Asia, and Central and South America, although sub-Saharan Africa is the most strongly affected. Efforts to reduce poverty and childhood mortality in those vulnerable societies will fail if this devastating disease is not adequately controlled" (President's Malaria Initiative [PMI], 2016).

"The spread and the emergence of resistance to the front-line antimalarial drugs including artemisinin is the major challenge that jeopardizes all recent gains in malaria control and has major implications for public health" (Ashley et al., 2014). "The scientific community is now underway to combat this problem by searching for new, affordable and effective antimalarial agents from medicinal plants and other sources" (Gamo, 2014). "Historically, the majority of conventional antimalarial drugs have been derived from plants or structures modeled on plant-derived compounds and over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs of the pharmaceutical industry" (Erhirhie et al., 2021). "Traditional healers have long used plants to prevent or cure infections. Herbal medicine or phytomedicine refers to the use of any plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which are based on their use in traditional medicine. It has

been noted that the source of many important pharmaceuticals raw materials has been plants used by indigenous people" (Aziz et al., 2018).

"Countries in Africa, Asia and Latin America use herbal and traditional medicine to help meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. In industrialized countries, the adoption of traditional medicine termed ''Alternative medicine'' is getting much higher attention in recent years. Research shows medicinal plants continue to play an important role in health aid" (Erhirhie et al., 2021). "*In vivo* antimalarial tests generally assess the development of rodent specific parasites *P. berghei*, *P. yoelii*, and *P. chabaudi* in mice, after subcutaneous and/or oral administration. Activity is expressed as a decrease of parasitemia after a certain time which is examined in smears or as survival time. These parasite models are indispensable for the development of antimalarials even if they do not always perfectly mirror *P. falciparum* infection in humans. In-vivo models are usually applied in antimalarial studies since they allow the possible prodrug effect and likely boosting of the immune system of the body against the pathogen" (Kifle & Atnafie, 2020). Despite widespread development of resistance, currently used and potent antimalarial drugs such as artemether, chloroquine and quinine are obtained from plant sources. Hence, it is imperative to research traditionally used medicinal plants such as *G. kola* for the discovery of possible new innovative antimalarial sources for the future.

2. METHODOLOGY

2.1 Description of Study Area

The fresh nut and leaf of *Gacinia kola* plant were collected based on Ethnobotanical description and with the help of taxonomist and local traditional healers in their natural habitats in Kurmi LGA of Taraba State. Kurmi is located between latitude 6^0 30' and 9^0 36'N and longitude 9^0 10' and 11^0 50'E. Kurmi is bounded in the West by Donga and Takum LGA and on the East by Gashaka LGA. It is bounded by Bali LGA on the Northern part, Ussa LGA on the Western part and Sardauna LGA on the Southern part. The climatic weather is wet and there is dry and rainfall season. The soil is generally sandy-loam. Soil color ranges from grayish-brown to brown and it is well drained. It is a high forest region with dense grasses and many tall trees. Most of the residents of Kurmi are Tigun, Ndola and Ichen by tribe and majority of them are farmers.

2.2 Experimental Design

The fresh nut and leaf of *G. kola* plant were collected, washed and air dried then packaged. Male and Female (non-pregnant) Mice of bodyweight 20 g to 35 g were purchased at Animal House, National Veterinary Research Institute Vom, Plateau State. The Mice were allowed to acclimatize in the Infectious Diseases Research Laboratory, Modibbo Adama University, Yola. The acclimitzation was done for fourteen days during which they were fed with standard rodents' feed (Finisher) and tap water. Then, the mice were equally divided (5 mice/group). And the average weights of the Mice in the test group were measured and used to calculate the dosage of plant extract to be administered to the mice.

A total of 70 Mice was used for all the two different crude extracts used in the curative test. The Mice were grouped into seven groups each containing five Mice. However, a total of 40 Mice were used for all the two different crude extracts used in the prophylactic test. The Mice were grouped into four groups each containing five Mice. In all cases of the plant extract, administration was done by compulsory oral intubations with the aid of cannula and syringe. The caring and experimental use of the Mice during this experiment was done using the guideline recommended by the Center for Drug Evaluation and Research (Center for Drug Evaluation & Research [CDER], 2011). Parasitemia for both curative and prophylactic test, MST of the experimental animal were all observed and recorded.

2.2.1 Collection and preparation of plant materials

The fresh leaf and stem-bark of *G. kola* plants were collected based on Ethnobotanical description and with the help of taxonomist. The fresh plant samples collected were identified and authenticated with a voucher number 02380 by a Taxonomist with the Department of Plant Science, Ahmadu Bello University Zaria. And the plants' samples were cleaned from extraneous materials by washing it carefully with clean water, air-dried under a shade at room temperature then cut and reduced to appropriate size. Thereafter, they were manually ground to powder with mortar and pestle. The powdered preparations were kept in a sterile plastic dish for further use. The plant screening test was conducted by a Laboratory technologist in the Chemistry Lab of Ahmadu Bello University Zaria, Kaduna State.

2.2.2 Ethanol extraction of plants

Powdered (100 g) of the *G. kola* plant samples were macerated with 100 ml of 80% ethanol for 72 hours with intermittent agitation. The supernatant part of agitated material filtered with 15 cm Whatman grade1 filter paper two times. The filtrate of *G. kola* plant samples were then concentrated using Rotary evaporator (BUCHI R-250, Switzerland) at 40^{o}C to remove Ethanol. The dried extract were kept at −20°C until used.

2.2.3 Source of experimental parasite

The *Plasmodium berghei* clones used in this study were obtained from the National Institute for Pharmaceutical Research and Development, Abuja. The parasites were chloroquine-sensitive ANKA clones phenotypes and were maintained by serial passage in Mice intraperitoneally by using a hypodermic needle.

2.3 Qualititative Phytochemical Screening Tests

Ethanol extracts of *G. kola* were screened for the presence of secondary metabolites to the antimalarial activity of the plant. Thus, tests for alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, anthraquinones and cardiac glycosides was performed in Chemistry Laboratory, Ahmadu Bello University Zaria; using standard test procedures reported by (Gavamukulya et al., 2014).

2.4 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was carried out to determine the quantity of alkaloids, tannins, saponin, flavonoids, phenols and terpenoids

2.5 Antimalarial Activity Testing

2.5.1 Inoculation of mice with parasites

P. berghei was obtained from a donor mouse. The parasitemia of the donor mice was first determined then the blood was collected and diluted with 0.8% normal saline based on parasitaemia level of the donor mice and the red blood cell count of normal mice, in such a way that 1ml blood contains 5 ×10^7 infected red blood cells of *P. berghei* infected erythrocytes. Hence every 0.2 ml of the aliquot could contain about 1x10^7 *P. berghei* infected red blood cells. Each mouse that was used in the experiment was inoculated with 0.2ml of infected blood containing about 1x10^7 *P. berghei* ANKA strain parasitized erythrocytes intraperitoneally by using a hypodermal needle.

2.5.2 Four-days suppressive test for Curative groups

Selected male mice weighing 20 g to 35 g were infected with 1x10^7 infected RBC (infected with *P. berghei*) and randomly divided into respective groups of five mice per cage. The mice were randomly divided into seven groups as follows:

i. Group A was infected with Malaria parasite at day 0 and was treated daily with 200mg/kg of extracts from day 4 to day 8;
ii. Group B was infected with malaria parasite at day 0 and was treated daily with 400mg/kg of extracts from day 4 to day 8;
iii. Group C was infected with malaria parasite at day 0 and was treated daily with 600mg/kg of extracts from day 4 to day 8;
iv. Group D (positive control) was infected with malaria parasite at day 0 and was treated daily with 200mg/kg of standard drugs (in this case choroquine) from day 4 to day 8;
v. Group E (positive control) was not infected but was given 200mg/kg of the extract daily from day 0 to day 8;
vi. Group F (negative control) was infected with malaria parasite at day 0 and was not treated;
vii. Group G (normal control) was not infected with the parasite and was not given the extract.

All the extracts were given through the intragastric route using a standard intragastric tube to ensure safe ingestion of the extracts and the drug (Center for Drug Evaluation & Research [CDER], 2011). Treatment started on day 4 and continued daily for four days (i.e. from day 4 to day 8) and blood samples were collected at day 9, and it was examined for parasitemia.

2.5.3 Four-days suppressive test for chemoprophylactic effect

Evaluation of the prophylactic potential of the extract was done using Peters methods with slight modification (Peters, 1967). The mice were grouped into four groups each containing five mice. For both *G. kola* plant parts ethanolic extracts prophylactic test, the grouping was as follows:

i. Group A was given 200mg/kg of *G. kola* nut extract daily from day 0 to day 4 and was infected with malaria parasite at day 5;
ii. Group B was given 200mg/kg of *G. kola* leaf extract daily from day 0 to day 4 and was infected with Parasite at day 5;

iii. Group C (positive control) was given 200mg/kg of standard drugs (in this case choroquine) daily from day 0 to day 4 and was infected with malaria parasite at day 5;
iv. Group D (negative control) was infected with malaria parasite at day 0 and was not treated.

Blood samples were collected from all the mice in the different groups at day 9, and it was examined for parasitemia. PCV, Body weight, rectal temperature were examined at pre-infection (PI) day, day 4 and day 9 for all the groups.

2.5.4 Microscopic examination of the parasite

On the 9th day (Day-9), that was 24 hours after the last dose, a blood sample was collected from a tail snip of each mouse (Parasuraman et al., 2010). The smears were applied on microscope slides, fixed with absolute methanol for 15 minutes and stained with 15% Giemsa stain at pH 7.2 for 15 minutes. The stained slides were then washed gently using distilled water and air-dried at room temperature. Each stained slide was then examined under a microscope with an oil immersion objective of 100X to evaluate the percentage suppression of each extract for the treated and control group. The parasitaemia level was determined by counting a minimum of five fields per slide with 100 RBC in a random field of the microscope. Percentage parasitaemia and the percentage of suppression were calculated and recorded.

2.6 Determination Mean Survival Time

2.6.1 Determination of mean survival time (MST)

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow-up period (D0 to D29). The mean survival time (MST) for each group was calculated using the formula described as follows:

MST= Sum of time (days) of all mice in group/Total number of mice in that group

2.7 Statistical Analysis

Results were analyzed using SPSS version 24. Comparisons were made between negative control, positive control (chloroquine) and treatment groups of various doses. The significance of disparity was determined using a 1-way analysis of variance (ANOVA) and the quantity of the phytochemical quantitative analysis was expressed as Mean ± Standard Error Mean of 3 replicates while the % Parasitemia were analysed and expressed as Mean ± Standard Error mean of 5 replicates, also the Mean Survival Time (MST was analyzed and recorded ± Standard Error mean of 5 replicates; Mean values with the same alphabets were not significantly different while Mean values with different alphabets were significantly different.

3. RESULTS AND DISCUSSION

Table 1. Phytochemical qualitative analysis of *G. kola* (nut and leaf) ethanolic extracts

Phytochemical	*Garcinia kola*	
	Leaf	**Nut**
Alkaloids	**+**	**+**
Flavonoids	**+**	**+**
Phenols	**+**	**+**
Saponins	**+**	**+**
Tannins	**+**	**+**
Terpenoids	**+**	**+**

Table 2. Phytochemical quantitative analysis of *G. kola* (nut and leaf) ethanolic extracts expressed as (mg/100 g)

Phytochemical	*G. kola*	
	Nut	**Leaf**
Saponins	2.70 ± 0.03^{a}	3.90 ± 0.02^{b}
Flavonoids	0.47 ± 0.01^{a}	1.38 ± 0.01^{b}
Tannins	0.72 ± 0.01^{a}	0.43 ± 0.01^{a}
Alkaloids	0.63 ± 0.03^{a}	2.87 ± 0.03^{b}
Phenols	0.13 ± 0.02^{a}	3.01 ± 0.04^{b}
Terpenoids	0.74 ± 0.03^{a}	2.64 ± 0.03^{b}

Table 3. Effect of *G. kola* nut ethanolic extract on % parasitemia and suppression in curative test groups

Group	Doses (mg/kg)	% Parasitemia	% Suppression	MST
Group A	200	28.30±0.78^{d}	40.14^{a}	12.42±0.20^{a}
Group B	400	24.54±2.13^{c}	45.S98^{b}	16.85±0.12^{b}
Group C	600	18.05±2.05^{b}	61.82^{c}	17.20±0.23^{b}
Group D	200	8.64±2.28^{a}	81.73^{d}	26.03±0.01^{c}
Group E	200	0.00	0.00	30.00±0.00^{c}
Group F	0	47.28±0.86^{e}	0.00	10.45±0.54^{a}
Group G	0	0.00	0.00	30.00±0.00^{c}

Values were expressed as Mean ± Standard Error mean of 5 replicates. Mean values with different supercripts in the same column differ significantly at p<0.05

Table 4. Effect of *G. kola* leaf ethanolic extract on % parasitemia and suppression in curative test groups

Group	Doses (mg/kg)	% Parasitemia	% Suppression	MST
Group A	200	31.40±0.78^{b}	33.78^{a}	10.92±0.41^{a}
Group B	400	30.14±1.01^{b}	36.44^{ab}	14.05±0.33^{b}
Group C	600	28.48±0.50^{b}	39.94^{b}	16.28±0.37^{b}
Group D	200	8.30±2.20^{a}	82.50^{c}	28.43±0.02^{c}
Group E	200	0.00	0.00	29.02±0.01^{c}
Group F	0	47.42±0.62^{d}	0.00	10.00±0.23^{a}
Group G	0	0.00	0.00	30.00±0.00^{c}

Values were expressed as Mean ± Standard Error mean of 5 replicates. Mean values with different supercripts in the same column differ significantly at p<0.05

Table 5. Effect of *G. kola* (nut and leaf) ethanolic extracts on % parasitemia and MST in prophylactic test groups

Group	Doses (mg/kg)	% Parasitemia	% Suppression	MST
Group A	200	21.96±1.29^{b}	54.63^{a}	16.25±0.34^{b}
Group B	200	24.98±0.53^{b}	48.39^{b}	14.90±0.40^{b}
Group C	200	3.70±2.28^{a}	92.36^{c}	30.00±0.00^{c}
Group D	0	48.40±0.61^{c}	0.00	10.48±0.52^{a}

Values were expressed as Mean ± Standard Error mean of 5 replicates. Mean values with different supercripts in the same column differ significantly at p<0.05

4. DISCUSSION OF FINDINGS

The present study revealed the phytochemical screening of major classes of compounds in *Garcinia kola* (nut and leaf) ethanolic extracts, where alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, and steroids were detected. These findings align with those of (Adebayo et al., 2014), who also reported similar phytochemical content in medicinal plants. The bioactive compounds found in *G. kola* may be responsible for its antimalarial activity, contributing to both therapeutic and prophylactic efficacy, as indicated in this study. Alkaloids, terpenoids, saponins, and flavonoids, among other compounds, have been shown to exhibit antiplasmodial activity through various mechanisms, corroborating findings by Tajuddeen & Van-Heerden, (2019). The choice of ethanol as the solvent in this study was influenced by previous reports, such as those by (Efe et al., 2017), which suggested that organic solvents extract a broader spectrum of bioactive substances compared to aqueous solvents. This implies that ethanol is a suitable alternative for evaluating the antimalarial properties of plants, as it can dissolve various chemical constituents more effectively.

In the curative test, *G. kola* ethanolic extract (nut and leaf) significantly reduced parasitemia, achieving parasitemia suppression of ≥30% within four days. This observation is consistent with the findings of (Krettli et al., 2009; (Madaki, 2015), who reported the efficacy of plant extracts in suppressing parasitemia. The suppression effect seen in *G. kola* may be attributed to its secondary metabolites, which are known for their

biological activities. These phytochemicals play significant roles in bioactivity, and their therapeutic value lies in the specific and definite actions they exert on the body. For instance, phenols, which possess antioxidant properties, may contribute to the antiplasmodial activity of *G. kola*. Antioxidants inhibit haem polymerization, which is critical in malaria pathology, as unpolymerized haem is toxic to intraerythrocytic plasmodia. Terpenoids and alkaloids have also been shown to inhibit haem polymerization through their binding with haemin. Additionally, flavonoids have been reported to inhibit the influx of L-glutamine and myoinositol into infected erythrocytes, further contributing to antimalarial activity. There was also a noticeable improvement in the physical activities of the infected mice treated with *G. kola* extracts compared to the untreated parasitized group. The treated mice displayed higher physical activity levels, which can be attributed to the parasitemia suppression effect of the extracts, leading to an overall improvement in their health condition.

In the prophylactic test, *G. kola* ethanolic extract demonstrated significant antimalarial activity, achieving a parasitemia reduction of ≥30% within four days, supporting the findings of (Krettli et al., 2009). The study also agrees with (Pradhan et al., 2011), who suggested that the prophylactic antiplasmodial activity observed in plant extracts could be due to the combined action of phytochemicals. In this study, alkaloids, saponins, phenols, and flavonoids in *G. kola* likely contributed to the observed prophylactic efficacy. Reports by (Saxena et al., 2013) have indicated the antimalarial roles of alkaloids, flavonoids, phenols, and saponins, further supporting the findings of this study. Additionally, the study is in line with that of (Petros & Daniel, 2012), who reported significant prophylactic effects of medicinal plant extracts against *Plasmodium berghei* at higher doses (600 mg/kg), as seen in this study with *G. kola*. The high efficacy of the plant extracts in the prophylactic test is also supported by (Jigam et al., 2012), who demonstrated that plant extracts inhibit parasite growth, thereby preventing anemia in malaria-infected subjects. These results highlight the potential of *G. kola* as a valuable resource for antimalarial therapies, both for treatment and prevention.

5. CONCLUSION

This study demonstrated that *Garcinia kola* (nut and leaf) ethanolic extracts possess significant antimalarial activity, both in curative and prophylactic tests. The phytochemical analysis revealed the presence of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids, which likely contribute to the observed antiplasmodial effects. The extracts showed dose-dependent efficacy in reducing parasitemia and improving the physical health of treated mice. These findings revealed the potential of *G. kola* as a natural source of antimalarial agents and validate its use in traditional medicine for malaria treatment and prevention.

6. RECOMMENDATIONS

i. Future studies should focus on isolating and characterizing the specific bioactive compounds in *G. kola* responsible for its antimalarial effects.
ii. Clinical trials should be conducted to evaluate the safety and efficacy of *G. kola* extracts in humans, particularly as a potential alternative or complementary treatment for malaria.
iii. Efforts should be made to develop pharmaceutical formulations, such as capsules or tablets, from *G. kola* extracts.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL CONSIDERATION

Approval for the study was obtained from Modibbo Adama University, Yola Ethics and Research Committee.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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