Research article

Anti-Malarial Bioactivity of *Garcinia kola* and *Vernonia amygdalina* Ethanolic Extracts in the Treatment of *Plasmodium berghei*-Infected Mice

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Malaria is one of the world's most serious infectious diseases caused by *Plasmodium* parasites. This research was aimed to determine the antimalarial bioactivity of *Gacinia kola* and *Vernonia amygdalina* ethanolic extracts in the treatment of malaria infection using an in vivo mouse model which was infected with *Plasmodium berghi*. The experiment was designed to assess the safety, the curative and prophylactic antimalarial activity of the individual extracts and the combined effect of the two extracts. Mice were evaluated using mean survival time, packed cell volume, rectal temperature and bodyweight. The percentage parasitemia suppression in mice treated with 200mg/kg, 400mg/kg and 600mg/kg of the *G. kola* nut ethanol extract demonstrated suppressive curative test was 40.14%, 45.98% and 61.82%, in four days, respectively. The statistical analysis indicates a significant difference when the mice were treated with different doses of the *G. kola* nut ethanolic extract. In comparison, the percentage parasitemia suppression in mice treated with 200mg/kg, 400mg/kg and 600mg/kg of the *V. amygdalina* leaf ethanolic extract suppressive curative test was 54.62%, 57.49% and 60.78%, in four days. However, there was no significant difference when the mice were treated with the different doses of the *V. amygdalina* leaf ethanolic extract. The study revealed that *G. kola* (nut and leaf), *V. amygdalina* (leaf and stem-bark) ethanolic extracts in the curative and prophylactic test group were effective in the treatment of malaria reducing the percentage parasitemia ≥30% in four days. This study observed that the extracts of *V. amygdalina* and *G. Kola* are potential sources of antimalarial compounds. Further evaluation of the clinical efficacy of these plant extracts in human volunteers is needed.

Keywords: Antimalarial activity, Efficacy, Ethanolic extracts, *Garcinia kola, Vernonia amygdalina*

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Introduction

Malaria remains one of the most serious global health problems, significantly contributing to morbidity and mortality, especially in endemic regions. In many African countries, malaria accounts for over 30% of outpatient visits and hospital admissions.[17] Despite efforts to control the disease, challenges remain due to the high costs of antimalarial drugs, which many Nigerians, particularly those in rural areas, cannot afford. Even for individuals who can afford these medications, safety concerns persist due to the increasing prevalence of drug-resistant strains of Plasmodium species, particularly to frontline antimalarial drugs like artemisinin derivatives.[2]

The management of malaria is further complicated by the absence of a clinically proven vaccine, insecticide resistance in mosquitoes, and the proliferation of fake drugs. In addition, current methods, such as indoor spraying with insecticides, are hampered by limited infrastructure and resources.[2] Moreover, artemisinin-resistant *Plasmodium falciparum* poses a significant threat to malaria control and elimination efforts.[2] Although chloroquine-resistant strains can still be treated with artemisinin derivatives, there is no approved alternative antimalarial drug to replace them if resistance becomes more widespread.

The impact of malaria extends beyond health, affecting the economy through lost working hours, healthcare costs, and reduced national productivity. Malaria is still endemic in over 100 countries, particularly in sub-Saharan Africa, Southeast Asia, and parts of Central and South America. Failure to adequately control malaria will undermine efforts to reduce poverty and childhood mortality in vulnerable communities.[17]

One of the most pressing challenges in malaria control is the emergence of resistance to frontline drugs, including artemisinin, which threatens recent progress in combating the disease.[2] In response, the scientific community is exploring new, affordable, and effective antimalarial agents from medicinal plants.[8] Historically, many conventional antimalarial drugs, such as artemether, chloroquine, and quinine, have been derived from plants or modeled on plant-derived compounds. Over 50% of modern clinical drugs have natural product origins, and medicinal plants play a crucial role in drug development.[7]

Traditional healers have long used plants to treat various infections, and herbal medicine, or phytomedicine, involves the use of seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. An impressive number of modern drugs have been isolated from natural sources, many of which were traditionally used by indigenous people.[3] In regions like Africa, Asia, and Latin America, herbal and traditional medicine is used to meet primary healthcare needs, with up to 80% of the population relying on traditional remedies. Even in industrialized nations, interest in "alternative medicine" has grown in recent years, with medicinal plants continuing to play an important role in health care.[3]

In vivo antimalarial testing typically involves rodent-specific parasites such as *Plasmodium berghei*, *P. yoelii*, and *P. chabaudi* in mice, which assess the development of parasitemia after treatment.[11] While these models may not perfectly replicate human *P. falciparum* infections, they are essential for developing antimalarial drugs. Researching traditionally used medicinal plants like *Garcinia kola* and *Vernonia amygdalina* is crucial for discovering new antimalarial compounds that could contribute to future treatment options.

Methodology

Description of Study Area

The fresh nut and leaf of *Garcinia kola* and *Vernonia amygdalina* plants were collected based on the ethnobotanical description and with the help of a 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 rainy seasons. 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 the majority are farmers.

Experimental Design

The fresh nut and leaf of *G. kola and V. amygdalina* plants were collected, washed and air dried then packaged. The *G. kola* and *V. amygdalina* plant samples collected were identified and authenticated with a voucher number 02380 and 02006 respectively by a taxonomist with the Department of Plant Science, Ahmadu Bello University Zaria. The plant screening test was conducted by a laboratory technologist in the chemistry lab of Ahmadu Bello University Zaria, Kaduna State. Male and female (non-pregnant) mice of bodyweight 20g to 35g 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 acclimatization was for fourteen days during which they were fed 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 140 mice were used used in the curative test. The mice were grouped into seven groups each containing five mice for the treatment group. While for the prophylactic group, the mice were grouped into four groups each containing five mice. In all cases of the plant extract, administration was performed by compulsory oral intubations with the aid of cannula and syringe. The caring and experimental use of the mice during this experiment was performed according to the guidelines recommended by the Center for Drug Evaluation and Research.[5]Parasitemia for both curative and prophylactic tests, synergism potentials of the plant extract, body weight, temperature and packed cell volume (PCV) of the experimental animals were observed and recorded.

Collection and Preparation of Plant Materials

The fresh leaf and stem-bark of *G. kola and V. amygdalina* plants were collected based on the ethnobotanical description and with the help of a taxonomist. The plant samples were cleaned from extraneous materials by carefully washing with clean water, air-dried under a shade at room temperature then cut and reduced to appropriate size. Following cutting the samples were manually ground to powder with a mortar and pestle. The powdered preparations were stored a sterile plastic dish for further use.

Ethanol Extraction of plants

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

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 clone phenotypes and were maintained by serial passage in mice intraperitoneally.

Qualitative phytochemical screening Tests

Ethanol extracts of *G. kola* were screened for the presence of secondary metabolites. Thus, tests for alkaloids, flavonoids, terpenoids,

phenols, tannins, saponins, anthraquinones and cardiac glycosides was performed in the chemistry laboratory, Ahmadu Bello University Zaria; using standard test procedures.[9]

Quantitative phytochemical Analysis

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

Antimalarial Activity Testing

Inoculation of Mice with Parasites and Extract administration

P. berghei was obtained from a donor mouse. The parasitemia of the donor mice was first determined. A blood sample was collected and diluted with 0.8% normal saline based on the level of parasitemia of the donor mice and the red blood cell count of normal mice, standardizing he volume of 1ml blood containing approximately 5 $\times 10^7$ infected red blood cells of *P. berghei*.. Hence every 0.2 ml of the aliquot should contain about $1x10^7$ *P. berghei* infected red blood cells. Each mouse that was used in the experiment was inoculated with 0.2ml of the infected blood sample containing about $1x10^7$ *P. berghei* ANKA strain intraperitoneally by using a hypodermal needle.

All the extracts were administered using a standard intragastric tube to ensure safe ingestion of the extracts and the drug.[5] Treatment started on day 4 and continued daily for an additional four days (i.e. from day 4 to day 8) and blood samples were collected at day 9, and examined for parasitemia. PCV, body weight, and rectal temperature were examined at pre-infection (PI) day, day 4 and day 9 for all the groups.

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

Microscopic Examination of the Parasite

On the 9th day (Day-9), 24 hours after the final dose of extract, a blood sample was collected from a tail snip of each mouse.[1] 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 examined under a microscope with the oil immersion objective of 100X to evaluate the percentage suppression of each extract for the treated and control groups. The parasitemia level was determined by counting a minimum of five fields per slide with 100 RBC in a random field of the microscope. Percentage parasitemia and the percentage of suppression were calculated and recorded.

Determination of Body Weight and Rectal Temperature

The body weight of each mouse in all groups was determined before infection on the first day or pre-infection day (24 hours before infection); on day 4 (post infection) and on day 9 (24 hours after treatment) using a sensitive weighing balance (METTLER TOLEDO, Switzerland). The rectal temperature of the mice was measured with a digital thermometer on pre-infection day (24 hours before infection); on day 4 (post infection) and on day 9 (24 hours after treatment). The % change in body weight and rectal temperature was calculated and recorded.

Determination of Packed Cell Volume (PCV)

Packed cell volume (PCV) was measured to predict the effectiveness of the test extract and fractions in preventing hemolysis resulting from increasing parasitemia associated with malaria. Blood was collected from the tail of each mouse in heparinized micro hematocrit capillary tubes. The capillary tubes were filled with blood up to 3/4th of their volume and sealed. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge (Hettich hematocrit, Germany) with the sealed ends outwards and centrifuged for 5 min at 11,000 rpm. PCV is a measure of the proportion of RBCs to plasma on pre-infection day (24 hours before infection); on day 4 (post infection) and on day 9 (24 hours after treatment).[15]

Ethical Considerations

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

Statistical Analysis

Results were analyzed using SPSS version 24. Comparisons were made between the negative control, positive control (chloroquine) and treatment groups at the 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 analyzed and expressed as Mean ± Standard Error mean of 5 replicates, also the mean survival time (MST), the PCV, rectal temperature and bodyweight differences were analyzed and recorded ± Standard Error of the mean of 5 replicates; Superscript alphabets such as [a] and [b] were used to represents the statistical difference, mean values with the same alphabets were considered not significantly different while mean values with different alphabets were significantly different.

Results

The phytochemical qualitative analysis of Garcinia kola (leaf and nut) ethanolic extracts revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids in both plant parts (Table 1). Similarly, Vernonia amygdalina (leaf and stem-bark) ethanolic extracts contained alkaloids, phenols, saponins, tannins, and terpenoids, but flavonoids were absent in the stem-bark (Table 2). The quantitative phytochemical analysis of G. kola revealed that the leaf extract contained higher concentrations of alkaloids (2.87 ± 0.03 mg/100g), flavonoids (1.38 ± 0.01 mg/100g), phenols (3.01 ± 0.04 mg/100g), saponins (3.90 ± 0.02 mg/100g), and terpenoids (2.64 ± 0.03 mg/100g) compared to the nut extract, while tannins were slightly higher in the nut extract (0.72 ± 0.01 mg/100g) (Table 3). For *V. amygdalina*, the leaf extract had a higher concentration of alkaloids (6.51 ± 0.03 mg/100g), flavonoids (2.87 ± 0.03 mg/100g), phenols (3.24 ± 0.03 mg/100g), saponins (4.32 ± 0.04 mg/100g), while the stem-bark extract contained higher levels of tannins (2.86 ± 0.01 mg/100g) and terpenoids (0.28 ± 0.02 mg/100g) (Table 4).

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

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

+ (Present) and - (Absent)

Table 2. Phytochemical qualitative analysis of *V. amygdalina* (leaf and stem-bark) ethanolic extracts

Phytochemical	*V. amygdalina*	
	Leaf	Stem-bark
Alkaloids	+	+
Flavonoids	+	-
Phenols	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+

+ (Present) and - (Absent)

Table 3. Phytochemical quantitative analysis of *G. kola* (leaf and nut) ethanolic extracts expressed as (mg/100g)

Phytochemical	*Garcinia kola*	
	Leaf	Nut
Alkaloids	2.87 ± 0.03[b]	0.63 ± 0.03[a]
Flavonoids	1.38 ± 0.01[b]	0.47 ± 0.01[a]
Phenols	3.01 ± 0.04[b]	0.13 ± 0.02[a]
Saponins	3.90 ± 0.02[b]	2.70 ± 0.03[a]
Tannins	0.43 ± 0.01[a]	0.72 ± 0.01[a]
Terpenoids	2.64 ± 0.03[b]	0.74 ± 0.03[a]

Values were expressed as Mean ± Standard Error mean of 3 replicates. Mean values with different supercripts in the same row differ significantly at $p<0.05$.

Values were expressed as Mean ± Standard Error mean of 3 replicates. Superscript alphabets such as [a] and [b] were used to represents the statistical difference, mean values with the same alphabets were considered not significantly different while mean values with different alphabets were significantly different.

Table 4. Phytochemical quantitative analysis of *V. amygdalina* (leaf and stem-bark) ethanolic extracts expressed as (mg/100g)

Phytochemical	*V. amygdalina*	
	Leaf	Stem-bark
Alkaloids	6.51 ± 0.03[a]	3.04 ± 0.03[b]
Flavoniods	2.87 ± 0.03[a]	2.43 ± 0.04[a]
Phenols	3.24 ± 0.03[a]	3.02 ± 0.04[a]
Saponins	4.32 ± 0.04[a]	2.67 ± 0.02[b]
Tannins	1.37 ± 0.04[a]	2.86 ± 0.01[b]
Terpenoids	0.46 ± 0.05[a]	0.28 ± 0.02[b]

Values were expressed as Mean ± Standard Error mean of 3 replicates. Superscript alphabets such as [a] and [b] were used to represents the statistical difference, mean values with the same alphabets were considered not significantly different while mean values with different alphabets were significantly different.

The curative effect of *G. kola* nut ethanolic extract on parasitized mice showed a decrease in packed cell volume (PCV) by day 4 post-infection, followed by a partial recovery by day 9. Mice treated with 600 mg/kg of the extract experienced a 20.30% improvement in PCV, whereas the untreated infected group (Group F) had a drastic reduction of 41.50% (Table 5). Similarly, body weight changes revealed a decline by day 4, with partial recovery by day 9 across treated groups, except for the untreated infected group, which exhibited a significant weight loss of 14.96% (Table 6). The rectal temperature of infected mice showed fluctuations, with some treatment groups exhibiting reductions, while others remained stable (Table 7). For *G. kola* leaf ethanolic extract, PCV also dropped by day 4 but showed recovery by day 9, with the highest improvement of 17.92% observed in the group receiving 200 mg/kg of the extract (Table 8). The weight of treated mice slightly declined by day 4, but by day 9, there was a moderate recovery in most groups, with the untreated infected group experiencing the highest weight loss of 12.17% (Table 9). The rectal temperature followed a similar trend, with some treated groups showing a slight decline and others stabilizing (Table 10).

Table.5. Effect of parasite and *G. kola* nut ethanolic extract on PCV of mice in the curative test groups

Group	Doses (mg/kg)	PCV (%)			% change in PCV
		PI	D4	D9	
Group A	200	42.66±1.28[a]	32.78±1.41[b]	36.63±1.63[b]	14.14
Group B	400	41.42±2.26[a]	33.16 ±0.89[b]	35.76±1.35[b]	13.66
Group C	600	42.66 ±0.70[a]	30.35 ±1.14[b]	34.00±0.90[b]	20.30
Group D	200	42.12±1.28[a]	25.62 ±0.73[d]	35.88±0.84[c]	14.15
Group E	200	41.72±0.84[a]	41.84±0.52[a]	41.92±0.55[a]	-0.48
Group F	0	42.46 ±0.65[a]	30.20 ±0.50[b]	24.84±0.62[d]	41.50
Group G	0	41.50±0.75[a]	41.76 ±0.81[a]	41.80±0.80[a]	-0.72

Note: Values were expressed as Mean ± Standard Error mean of 5 replicates. Mean values with different superscripts in the same column differ significantly at $p<0.05$. PI stand for (Pre-infection; 24 hours before infection); D4 (Day 4; Post-infection) and D9 (Day 9; 24 hours after treatment).

Table 6. Effect of parasite and *G. kola* nut ethanolic extract on body weight of mice in the curative test groups

Group	Doses (mg/kg)	Body weight (g)			% change in
		PI	D4	D9	Body weight
Group A	200	30.78±1.34[a]	27.90±1.08[a]	29.43±1.30[a]	4.39
Group B	400	31.78±1.25[a]	29.60 ±1.29[a]	30.80±1.12[a]	3.08
Group C	600	31.70±1.75[a]	29.80±1.59[a]	31.00±1.75[a]	2.21
Group D	200	30.00±0.60[a]	27.74±0.79[a]	28.92±0.53[a]	3.60
Group E	200	30.96±0.48[a]	31.00±0.18[a]	31.30±0.58[a]	-1.10
Group F	0	30.22±1.18[a]	27.10 ±1.49[a]	25.70±1.29[b]	14.96
Group G	0	30.80± 0.84[a]	29.48±0.43[a]	29.82±0.40[a]	3.18

Table 7. Effect of parasite and *G. kola* nut ethanolic extract on rectal temperature of mice in the curative test groups

Group	Doses (mg/kg)	Rectal temperature (°C)			% change in Temperature
		PI	D4	D9	
Group A	200	35.10±0.60[a]	36.50 ±0.35[a]	35.17±0.41[a]	-0.19
Group B	400	34.90 ±0.48[a]	36.56 ±0.79[a]	34.96±0.39[a]	-0.17
Group C	600	34.70±0.65[a]	38.40±0.49[a]	36.60±0.84[a]	-5.48
Group D	200	35.80±0.60[a]	7.84±0.49[a]	6.54±0.53[a]	-2.07
Group E	200	35.42±0.62[a]	37.30±0.76[a]	37.32±0.46[a]	-5.36
Group F	0	38.06±0.75[a]	38.98±0.21[a]	38.72±0.30[a]	-1.94
Group G	0	35.92±0.53[a]	36.50 ±0.58[a]	36.28±0.58[a]	-1.00

Table 8. Effect of parasite and *G. kola* leaf ethanolic extract on PCV of mice in the curative test groups

Group	Doses (mg/kg)	PCV (%)			% change in PCV
		PI	D4	D9	
Group A	200	40.60±0.48[a]	31.20±0.84[b]	33.78±0.37[b]	17.92
Group B	400	38.04±0.29[a]	28.44±0.95[b]	32.06±0.54[b]	15.72
Group C	600	42.12±1.00[a]	33.26±2.35[b]	37.80±1.20[b]	10.26
Group D	200	41.90±0.07[a]	31.38±1.77[b]	36.46±0.39[c]	12.98
Group E	200	42.86 ±0.79[a]	42.92±1.15[a]	41.88±0.94[a]	2.29
Group F	0	42.28±1.02[a]	31.26±1.55[b]	24.78±0.86[c]	41.39
Group G	0	42.56±0.61[a]	41.62±0.58[a]	41.50±0.40[a]	2.49

Table 9. Effect of parasite and *G. kola* leaf ethanolic extract on Body weight of mice in the curative test groups

Group	Doses (mg/kg)	Body weight (g)			% change in Body weight
		PI	D4	D9	
Group A	200	31.06±1.32[a]	28.08±0.90[a]	30.32±0.45[a]	2.38
Group B	400	29.36±1.26[a]	27.36±1.17[a]	28.70±1.18[a]	2.25
Group C	600	32.36±1.61[a]	30.16±2.80[a]	31.20±2.00[a]	3.58
Group D	200	30.46±1.44[a]	27.88±1.43[a]	29.18±1.27[a]	4.30
Group E	200	30.04±1.21[a]	30.16±1.64[a]	30.44±1.26[a]	-1.33
Group F	0	30.74±1.48[a]	28.54±1.39[a]	27.00±1.30[a]	12.17
Group G	0	29.62±0.73[a]	28.72±0.63[a]	28.04±0.51[a]	5.33

Table 10. Effect of parasite and *G. kola* leaf ethanolic extract on rectal temperature of mice in the curative test groups

Group	Doses (mg/kg)	Rectal temperature (°C)			% change in Temperature
		PI	D4	D9	
Group A	200	36.04±0.50[a]	38.04±0.06[a]	37.36±0.26[a]	-3.66
Group B	400	35.22±0.41[a]	37.14±0.18[a]	35.52±0.56[a]	-0.85
Group C	600	35.70±0.36[a]	8.50±0.19[a]	36.08±1.89[a]	-1.06
Group D	200	36.12±1.06[a]	38.78±0.44[a]	36.96±0.83[a]	-2.33
Group E	200	35.52±0.54[a]	7.24±0.43[a]	36.88±0.52[a]	-3.83
Group F	0	35.54±0.99[a]	39.94±0.28[a]	38.76±0.35[a]	-9.06
Group G	0	36.24±0.38[a]	36.44±0.14[a]	36.72±0.22[a]	-1.32

The curative potential of *V. amygdalina* leaf ethanolic extract was also assessed, where PCV decreased post-infection and improved after treatment, with the highest recovery of 15.60% observed at a 600 mg/kg dose. Meanwhile, the untreated infected group experienced a significant PCV reduction of 48.77% (Table 11). The body weight of treated mice showed minor fluctuations, while the untreated infected group lost about 11.92% of its body weight (Table 12). The rectal temperature of treated groups remained relatively stable, whereas the untreated infected group exhibited a notable decline (Table 13). Also, PCV levels declined significantly in infected untreated mice (Group F), with a 49.53% reduction, while

treatment with 200–600 mg/kg of *V. amygdalina* stem-bark extract resulted in a lower percentage decrease (Table 14). Similarly, body weight reductions were observed in all treated groups, but the untreated infected group (Group F) showed the highest weight loss of 9.02% (Table 15). Rectal temperature fluctuations followed a similar trend, where infected untreated mice exhibited the most significant drop in temperature (-8.47%), while treated groups maintained relatively stable temperature levels (Table 16).

Table 11. Effect of parasite and *V. amygdalina* leaf ethanolic extract on PCV of mice in the curative test groups

Group	Doses (mg/kg)	PCV (%)			% change in PCV
		PI	D4	D9	
Group A	200	43.72±0.65^{a}	33.30±0.48^{b}	37.06±0.54^{b}	15.23
Group B	400	42.48±0.74^{a}	31.96±0.53^{b}	35.90±0.56^{b}	15.49
Group C	600	42.70±0.57^{a}	32.86±0.70^{b}	36.04±0.72^{b}	15.60
Group D	200	43.08±0.61^{a}	31.22±0.75^{b}	37.12±0.75^{c}	13.83
Group E	200	42.00±0.61^{a}	42.14±0.54^{a}	43.18±0.38^{a}	-2.81
Group F	0	42.12±0.41^{a}	31.78±0.89^{b}	21.58±0.60^{c}	48.77
Group G	0	42.96±0.96^{a}	42.62±0.88^{a}	43.08±0.54^{a}	-0.28

Table 12. Effect of parasite and *V. amygdalina* leaf ethanolic extract on body weight of mice in the curative test groups

Group	Doses (mg/kg)	Body weight (g)			% change in Body weight
		PI	D4	D9	
Group A	200	29.79±0.89^{a}	28.18±0.74^{a}	29.44±0.90^{a}	1.31
Group B	400	31.47±1.12^{a}	29.76±1.17^{a}	30.47±1.05^{a}	3.18
Group C	600	30.14±1.44^{a}	28.39±1.47^{a}	29.67±1.25^{a}	1.56
Group D	200	29.62±1.42^{a}	27.47±1.41^{a}	28.47±1.21^{a}	3.88
Group E	200	29.80±0.83^{a}	30.33±1.26^{a}	30.87±1.03^{a}	-3.59
Group F	0	29.44±1.03^{a}	27.00±0.99^{a}	25.93±0.85^{a}	11.92
Group G	0	30.61±0.80^{a}	29.27±0.66^{a}	29.53±0.49^{a}	3.53

Table 13. Effect of parasite and *V. amygdalina* leaf ethanolic extract on rectal temperature of mice in the curative test groups

Group	Doses (mg/kg)	Rectal temperature (^{0}C)			% change in Temperature
		PI	D4	D9	
Group A	200	35.70±0.56^{a}	36.49±0.40^{a}	36.27±0.57^{a}	-1.59
Group B	400	35.17±0.31^{a}	36.74±0.24^{a}	35.27±0.41^{a}	-0.28
Group C	600	35.69±0.97^{a}	35.37±0.55^{a}	34.09±0.65^{a}	4.48
Group D	200	36.01±0.93^{a}	37.99±0.35^{a}	36.81±0.68^{a}	-2.22
Group E	200	35.46±0.48^{a}	37.89±0.68^{a}	36.81±0.23^{a}	-3.81
Group F	0	37.22±0.90^{a}	38.50±0.43^{a}	38.61±0.32^{a}	-3.73
Group G	0	36.32±0.34^{a}	36.84±0.16^{a}	36.63±0.23^{a}	-0.85

Table 14. Effect of parasite and *V. amygdalina* stem-bark ethanolic extract on PCV of mice in the curative test groups

Group	Doses (mg/kg)	PCV (%)			% change in PCV
		PI	D4	D9	
Group A	200	41.70±0.64^{a}	32.64±1.01^{b}	36.84±0.53^{b}	11.65
Group B	400	41.40±0.48^{a}	30.78±0.82^{b}	35.68±1.25^{b}	13.82
Group C	600	42.16±0.68^{a}	34.24±1.41^{b}	37.62±0.92^{b}	10.77
Group D	200	42.10±1.05^{a}	31.38±0.87^{b}	36.22±0.89^{c}	13.97
Group E	200	42.44±0.83^{a}	42.22±0.56^{a}	41.14±0.53^{a}	3.06
Group F	0	42.28±0.65^{a}	31.26±0.51^{a}	21.34±0.60^{d}	49.53
Group G	0	40.64±0.23^{a}	40.84±0.34^{a}	40.52±0.63^{a}	0.30

Table 15. Effect of parasite and *V. amygdalina* stem-bark ethanolic extract on body weight of mice in the curative test groups

Group	Doses (mg/kg)	Body weight (g)			% change in Body weight
		PI	D4	D9	
Group A	200	30.93±0.12[a]	27.78±0.95[a]	29.40±1.33[a]	4.95
Group B	400	28.77±1.18[a]	26.61±1.14[a]	27.89±1.21[a]	3.06
Group C	600	32.00±1.39[a]	29.76±1.26[a]	30.83±1.41[a]	3.66
Group D	200	29.86±0.63[a]	27.12±0.78[a]	28.53±0.59[a]	4.45
Group E	200	29.14±0.47[a]	29.22±0.55[a]	29.50±0.47[a]	-1.22
Group F	0	30.38±1.06[a]	28.22±1.35[a]	27.64±1.85[a]	9.02
Group G	0	28.84±0.72[a]	28.38±0.41[a]	28.02±0.35[a]	2.84

Table 16. Effect of parasite and *V. amygdalina* stem-bark ethanolic extract on rectal temperature of mice in the curative test groups

Group	Doses (mg/kg)	Rectal temperature (°C)			% change in Temperature
		PI	D4	D9	
Group A	200	36.00±0.53[a]	37.89±0.25[a]	37.24±0.36[a]	-3.44
Group B	400	35.71±0.42[a]	37.54±0.59[a]	35.93±0.36[a]	-0.62
Group C	600	35.93±0.56[a]	38.60±0.42[a]	36.24±0.46[a]	-0.86
Group D	200	36.16±0.52[a]	38.69±0.50[a]	36.98±0.55[a]	-2.27
Group E	200	35.78±0.38[a]	37.46±0.12[a]	37.23±0.26[a]	-4.05
Group F	0	35.42±0.47[a]	38.90±0.22[a]	38.42±0.30[a]	-8.47
Group G	0	35.91±0.48[a]	36.39±0.52[a]	36.52±0.54[a]	-1.70

The parasitemia suppression in infected mice treated with *G. kola* ethanolic extracts and standard drugs varied across different concentrations. Treatment with 400 mg/kg of *G. kola* nut ethanolic extract resulted in the highest parasitemia suppression of 45.98%, which was significantly higher than the combination of 200 mg/kg of *G. kola* nut extract with 200 mg/kg of *G. kola* leaf extract (40.69%). Meanwhile, treatment with 400 mg/kg of *G. kola* leaf extract alone yielded a lower suppression rate of 36.44% (Figure 1). Similarly, the parasitemia suppression in mice treated with *V. amygdalina* ethanolic extracts

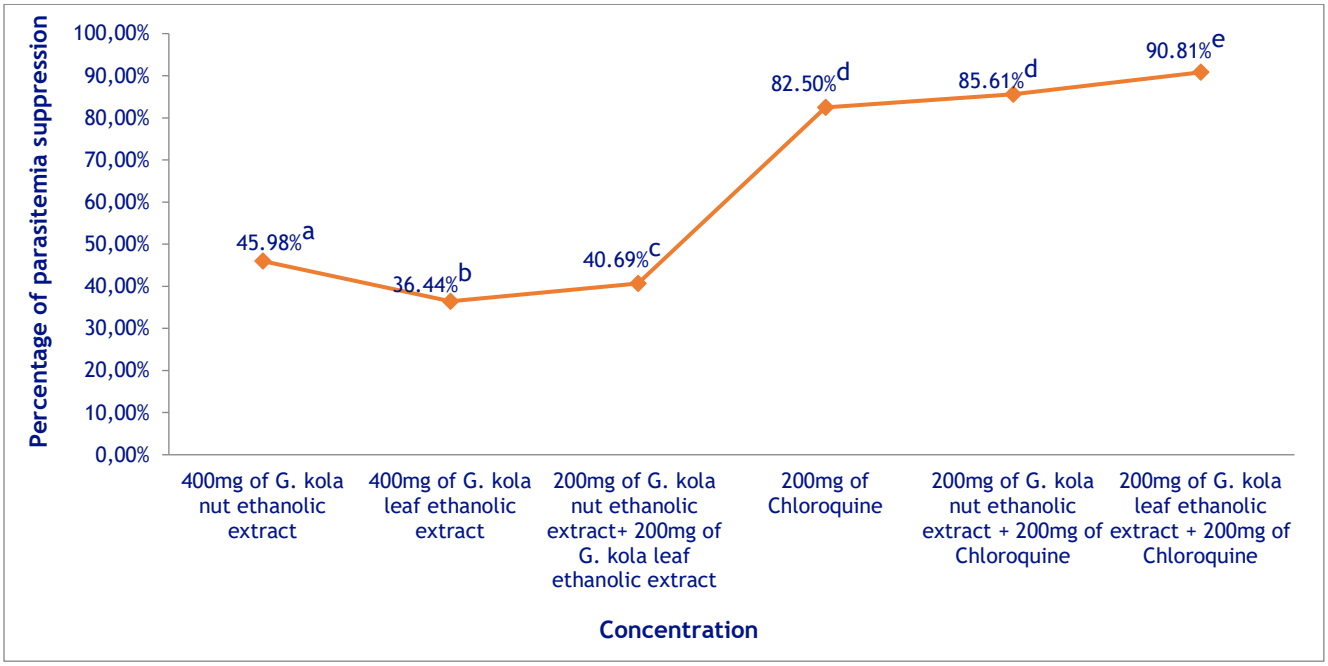

Figure 1. Percentage parasitemia suppression in infected mice treated with single and combined doses of *G. kola* ethanolic extracts and standard drug

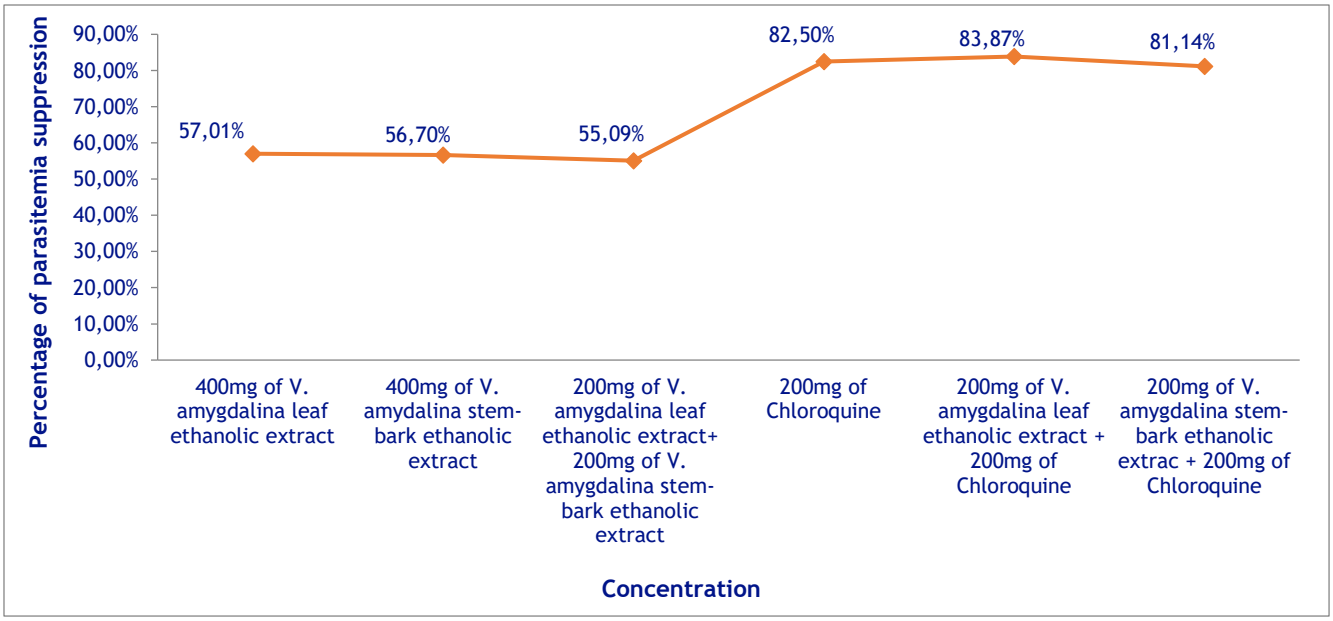

Figure 2. Percentage parasitemia suppression in infected mice treated with single and combined doses of *V. amygdalina* ethanolic extracts and standard drug

showed a slight variation in effectiveness. Administration of 400 mg/kg of *V. amygdalina* leaf extract resulted in 57.01% suppression, which was insignificantly higher than the combination of 200 mg/kg of *V. amygdalina* leaf extract with 200 mg/kg of *V. amygdalina* stem-bark extract (55.09%). Additionally, treatment with 400 mg/kg of *V. amygdalina* stem-bark extract produced a suppression rate of 56.09%, which was also insignificantly higher than the combined 200 mg/kg leaf and stem-bark extract treatment (55.09%) (Figure 2).

Discussion

The present study examined various major classes of phytochemical compounds; it was determined that alkaloid, flavonoids, phenols, saponins, tannins, terpenoids and steroids were present in *G. kola* (nut and leaf), and *V. amygdalina* (leaf and stem-bark) ethanolic extract. These bioactive substances may be responsible for antimalarial activity; the therapeutic and prophylactic efficacy of the two plants evaluated in this research.[1] The two plants possess different classes of phytochemicals such as alkaloids, terpenoids, saponins, and flavonoids and demonstrated anti-plasmodial activity through various mechanisms as previouslyreported.[18] The choice of using organic (ethanol) solvent in this study was based on reports that organic solvents yield more bioactive substances than the aqueous extraction due to an increase in solubility in organic solvents.[6] This implies that organic solvents are good alternatives in evaluating antimalarial plant properties as they can extract a wide spectrum of chemical constituents.

The result of the study showed a significant reduction on the PCV of the infected experimental mice in the curative test groups when observed at day four after infection. After infecting mice with malaria, the host (mouse) demonstrates a reduction in PCV. The underlying cause of this PCV reduction could be loss of infected erythrocytes through parasite maturation, destruction of uninfected red cells in the spleen and liver by macrophage activation and enhanced phagocytosis, reduced erythropoiesis and dyserythropoiesis. However, there was an increase in PCV when extracts at varying concentrations of the four different extracts was administered to the curative test group for four days after infection. This implies that the extract significantly prevented PCV reduction when compared to the negative control, as seen in previous studies.[4,15]

Furthermore, it was observed that there were no reasonable changes in the level of PCV in experimental mice in the prophylactic test group when the mice were not infected with parasites but were administered an extract for four days at a varying concentration of the different extracts used for this study. Unlike the curative group, on the other hand, the prophylactic model did not prevent a reduction in PCV, but the extract-treated group exhibited an improvement in prevention against PCV reduction. This agrees with the findings that ethanolic extracts do not prevent reduction PCV values.[15] However, the finding of this study differed from the research which indicated that the mice treated with the highest dose of the extract (600mg/kg) and the group administered the standard drug (Chloroquine) showed a high increase in PCV on the fifth day.[12] The difference in this finding could be attributed to the differences in the administered concentration of the extracts.

Body weight is another feature used to assess malaria infection in experimental mice. The result of the study showed a slight decrease in the body weight of the infected experimental mice in the curative test groups when observed for four days after infection. Body weight loss in extract-treated mice might be due to a depressing effect as a result of the increment of parasitemia. However, based on the result of the present study, there was a slight increase in body weight when extracts at varying concentrations of the four different extracts were administered to the curative test group for four days following infection with parasites. Even though, the bodyweight gained in the curative test animals for all doses, this could be due to the effect of the ethanolic extract which decreases the parasitemia on established infection since the inoculum was given three days prior to treatment. The finding of this study is similar to previously reported reported that mice treated with crude extracts showed a lower body weight reduction as compared with the non-treated.[12]

Rectal temperature is also an important feature used to assess malaria infection in experimental mice. The result of the study showed a slight increase in the rectal temperature of the infected experimental mice in the curative test groups when observed four days after infection. The finding in this study is related to the finding reported that the extract-treated mice demonstrate an increase in the prevention on rectal temperature reduction than normal control animals even though it was not statistically significant.[1] Active compounds should be able to prevent the rapid dropping of rectal temperature because decrease in internal body temperature will reduce the metabolic activity of the laboratory mice.

Furthermore, the result of this study revealed that there were no reasonable changes in rectal temperature of the experimental mice in the prophylactic test group when the mice were not infected with parasite but were administered with the four different extracts. This implies that the different doses (200mg/kg, 400mg/kg and 600mg/kg) of the two plant extracts (*G. kola* and *V. amygdalina*) significantly protected the decrease in rectal temperature associated with *P. berghei* infection in mice. This indicates that the extracts prevent some pathological processes that can cause the reduction in internal body temperature. Anemia, body weight loss and body temperature reduction are the general features of malaria infected mice. An ideal antimalarial agent obtained from a plant is expected to prevent anemia, body weight loss and regulate temperature in infected mice.

The result of this study revealed that 200mg/kg of *G. kola* nut + 200mg/kg of *G. kola* leaf ethanolic extracts demonstrated a higher percentage of parasitemia suppression than when 400mg/kg of *G. kola* leaf extract was administered alone. Chloroquine administered at 200mg/kg demonstrated a higher percentage parasitemia suppression when in combination with 200mg/kg *G. kola* leaf extract compared to when chloroquine was administered at 200mg/kg alone. The finding of this

study differs from the report that indicated chloroquine is more effective in combination with extract than independently.[10] The possible reason for the difference may be, the use of an aqueous extract of the plant used as an adjuvant with chloroquine. In the present study, the solvent used for the plant extraction was ethanol. Combination therapies are a vital strategy to prevent or delay resistance of parasites and have been approved for other multidrug-resistant infections.

The finding of this study revealed that there were no reasonable changes in the level of percentage parasitemia suppression when 400mg/kg of *V. amygdalin* leaf extract, 400mg/kg of *V. amygdalina* stem-bark extract and 200mg/kg of *V. amygdalina* leaf extract in combination with 200mg/kg of *V. amygdalina* stem-bark extract was administered to the infected mice. This study differs from previous studies that indicated very high parasite growth inhibition at two different doses (200mg/kg and 200mg/kg) of the combined extract of *C. citratus* and *V. amygdalina*. The reason for the difference may be due to the individual or synergistic effect of the two different plants.[14] The result of this study revealed that combining two or more plant parts did not connote higher efficacy compare to when only a single part was used. However, in some situations combining two or more plant parts demonstrated higher efficacy compared to when only a single part was used. These two scenarios indicate that combination therapy of the plants extract provided a synergistic reactions and antagonistic reactions in different scenarios.

Conclusion

This study indicates that the extracts of *V. amygdalina and G. kola* are potential sources of antimalarial compounds. It was observed that the plant extracts used in these studies had a curative antimalarial effect attributed to the presence of bioactive compounds present in the plants. It was also demonstrated that the plant extracts prevent anemia, body weight loss, regulate temperature and improve the mean survival time of the infected mice. Hence this implies that the extract has an important ingredient that is needed for the treatment of malaria. Anemia, body weight loss and body temperature reduction are the general features of malaria. The results of this study could help encourage more identification and validation of natural products, thus facilitating the development of a new generation of antimalarial drugs. Collaboration between the natural product scientists and the traditional healers could be of immense help and assist in the administration of the right doses of antimalarial compounds to avoid the risk of toxicity that may result from these herbal remedies.

Recommendations:

i. There is a need to promote the agricultural production of *G.kola* and *V. amygdalina* plants.
ii. Improve collaboration between traditional medicine and modern medicine.
iii. There is a need to repeat similar studies in a complex immune-compromised mouse model that has been developed to sustain *P. falciparum*-parasitized human erythrocytes *in vivo*.
iv. Evaluation of the *G.kola* and *V. amygdalina* plant extracts on different *Plasmodium* species and animal models are needed to better identify potential antimalarial activity.
v. Further pharmacological screening with bioassay-guided chemical fractionations of the crude extracts should be conducted to isolate the specific active ingredients responsible in the plant.
vi. Elucidating the structure and mechanism of action of the active ingredients of *G.kola and V. amygdalina* plant extracts is also recommended.
vii. There is a need to determine the clinical efficacy of *G.kola* and *V. amygdalina* plant extracts in human volunteers.

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