*FUDMA Journal of Sciences (FJS)*
*ISSN online: 2616-1370*
*ISSN print: 2645 - 2944*
*Vol. 8 No. 3, June, (Special Issue) 2024, pp 477 - 486*
*DOI: https://doi.org/10.33003/fjs-2024-0803-2502*

# ANTIMALARIAL ACTIVITY OF DECOCTION FROM *CARICA PAPAYA, PSIDIUM GUAJAVA* AND *MANGIFERA INDICA* LEAVES IN MICE INFECTED WITH *PLASMODIUM BERGHEI* (NK-65)

**Bashir, A., Bello, M. I., Mahmoud, S. J. and *Zailani, A. H.**

Department of Biochemistry, Faculty of Science, Modibbo Adama University, Yola, Adamawa State, Nigeria.

*Corresponding authors' email: hauwa.zailani@mau.edu.ng

## ABSTRACT

Malaria remains among the most common illnesses caused by parasites of the *Plasmodium spp*. It still poses a serious risk to millions of people living in both tropical and subtropical regions, causing thousands of deaths. The antimalarial activity of decoction from *Mangifera indica, Carica, and Psidium guajava* leaves in *Plasmodium berghei* inoculated mice was evaluated. Forty-nine mice averaging 22 g were grouped into 7 groups (7 per group). Group 1 served as naïve control, Groups 2–6 received intraperitoneal injections containing 1.0 x $10^7$ red blood infected with *Plasmodium berghei*. Administration of 100, 150, and 300 mg/kg body weight of the decoction to groups 4, 5, and 6 respectively was done daily after infection with the parasite was confirmed. Group 2 received no treatment, while Group 3 received four days of treatment with 20 mg/kg body weight of chloroquine. Group seven was decoction control (300 mg/kg body weight). The mice were sacrificed after the treatment and blood and liver tissue were collected for analysis. Phytochemical constituents of the decoction and its $LD_{50}$ were also determined. The decoction was found to contain appreciable amounts of phenolics, tannins, and alkaloids along with other phytochemicals and the calculated $LD_{50}$ was 5720 mg/kg body weight. Treatment with the decoction caused substantial decrease ($p < 0.05$) in parasite count within groups that received treatment compared to untreated ones and significantly ($p < 0.05$) improved biochemical parameters altered by malaria. The findings of this study revealed that the decoction is effective in management of malaria.

**Keywords**: Antimalarial, *Carica papaya, Psidium guajava, Mangifera indica, Plasmodium berghei*

## INTRODUCTION

Millions of people in tropical and subtropical nations continue to be at risk from malaria, one of the most common parasitic infections caused by the *Plasmodium* species. Ranging from 229 million cases and 409,000 deaths in 2019, it affected 247 million people worldwide and resulted in 619,000 deaths in 2021 (WHO, 2020; 2022). The COVID-19 pandemic-related shift in priorities and disruption of medical facilities were held accountable for this resurgence. Malaria is disproportionately concentrated in the African region which has about 94% of the disease burden. This is due to the abundance of the vector, *Anopheles gambiae* which fuels transmission. The majority of the people affected are children under the age of five and account for approximately 80% of all malaria deaths (WHO, 2022). Malaria has a huge socio-economic impact, and its elimination is a top priority worldwide. The pathogen or the vector are typically the targets of the fight against malaria, necessitating the development of novel and creative strategies to quicken the disease's elimination process. However, this has been very slow in Africa where the disease is prevalent. Moreover, global eradication strategies have been hampered by the emergence of insecticide-resistant vectors (Suh *et al*., 2023), multidrug-resistant parasite strains (Hamilton *et al*., 2019), and frequently changing global climatic conditions which affect vector population and transmission dynamics (Rocklov and Dubrow, 2020; Sinka *et al*., 2020).

In Nigeria, resistance to chloroquine and other anti-malarial drugs have been reported and over $1 billion is spent annually in treating the infection (Oche *et al*., 2016). Access to genuine, conventional antimalarial drugs in Nigeria is limited due to cost, lack of awareness, and cultural practices.

Additionally, the *Plasmodium* parasite has become resistant to some orthodox medications, which has made the search for new and more potent agents—particularly those derived from natural resources—necessary. For millennia, traditional and folkloric medicine has employed medicinal plants and their derivatives to treat a wide range of ailments, including malaria. It has been reported that around 80% of the global population still receives primary medical care from plants and up to 85% of people in Africa still use traditional herbal medication because of their affordability and accessibility and for their perceived safety (Uzo *et al.*, 2020; Singh *et al*., 2020).

*Carica papaya* (pawpaw) is a fruit widely grown in tropical, sub-tropical, and other regions, including Hawaii, Malaysia, Nigeria, Australia, Brazil, China, and India (Eziuche *et al*., 2023). It has been reported to contain several phytochemicals and has ferric-reducing antioxidant properties (FRAP), (Ghaffarilaleh *et al.*, 2019). Three essential vitamins with antioxidant potentials have been found in the pulp along with several minerals (Amin *et al*., 2019). Pawpaw also contains the important enzymes papain, (Amin *et al*., 2019) and chymopapain (Santana *et al*., 2019).

*Psidium guajava* (Guava) is a tropical plant mainly cultivated for fruit. It belongs to the family *Myrtaceae*. Guava leaves contain saponins, flavonoids, terpenes, and oleanolic acid (Naseer *et al.*, 2018). Antioxidants, polyphenols, antiviral, antibacterial, and anti-inflammatory compounds are among the many organic and inorganic secondary metabolites found in guavas along with antimicrobial and anti-inflammatory compounds.

A significant fruit from South and Southeast Asia, the mango (*Mangifera indica L.*) is a member of the *Anacardiaceae* family (Manoj *et al.*, 2021). Mangiferin, benzophenones, phenolic acids, and other phytochemicals, along with other antioxidants including ascorbic acid, carotenoids,flavonoids, and tocopherols, have all been related to the health benefits of mango leaves (Manoj *et al*., 2021). The biological actions of mango leaf extracts, such as lipid-lowering, hepatoprotection, antibacterial, antiobesity, anticancer, and anti-diarrheal properties, have been investigated (Manoj *et al.*, 2021).

Drug combination therapies are deemed vital to the most effective management of malaria since they have been shown to improve efficacy mostly through synergistic activities (Sadeghi *et al.,* 2020). Therefore, medication combination therapy, which uses polyherbal medicines made of two or more plant species or their parts that complement each other, has grown to be the accepted approach to treating illnesses in several parts of the world (Chaniad *et al.,* 2024). The antimalarial activity of the individual leaves used in this study and some of their combinations have been reported, but information about the efficacy of this specific decoction which is widely used by the local population in some parts of Northern Nigeria and in Adamawa State, Nigeria in particular and which the locals believe to be more effective in the treatment of malaria than the available conventional antimalarial drugs have not been reported. Therefore, the purpose of this research is to determine the impact of the decoction of the leaves of *Carica papaya, Psidium guajava*, and *Mangifera indica* on mice inoculated with *Plasmodium berghei*.

## MATERIALS AND METHODS

### Materials

#### *Plant Material*

Fresh leaf samples of *Carica papaya, Psidium guajava,* and *Mangifera indica* were obtained from a farm in Girei Local Government of Adamawa State and identified at the Department of Plant Sciences, Modibbo Adama University, Yola, Adamawa State.

#### *Laboratory Animals*

Mice weighing an average of 22 g gotten from the National Veterinary Research Institute, Vom, Plateau State were the laboratory animals used in this work. Before the experiment, they were acclimated to laboratory conditions (25 ˚C, 12 hr light /dark cycle) for 2 weeks during which they were maintained on commercial rat pellets (UAC Grand Cereal Ltd., Jos, Nigeria) and water *ad libitum*.

#### *Malaria Parasite*

*Plasmodium berghei* (NK-65) which is a chloroquine-strain was used to inoculate mice in this study. The parasite was collected from the Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria.

### Methods

#### *Preparation of the decoction*

Ethnobotanical information on the preparation of the decoction which consists of *Carica papaya* (Pawpaw), *Psidium guajava* (Guava), and *Mangifera indica* (Mango) leaves was obtained from an herbalist in Yola South L.G.A, Adamawa State, Nigeria. To make the decoction, one hundred and fifty (150) grams of *Carica papaya*, one hundred (100) grams of *Psidium guajava*, and fifty (50) grams of *Mangifera indica* leaves were collected, washed, and boiled together. The decoction was allowed to cool down. The concentration of the leaves in the decoction was recovered by lyophilization (Ćujić *et al*., 2016). The recovered yield was diluted in sterile distilled water to make up the doses administered in this study.

#### *Phytochemical screening*

Both qualitative and quantitative phytochemicals were screened for in the lyophilized decoction using standard methods of Trease and Evans 1989.

#### *Test for Median Lethal Dose ($LD_{50}$)*

This was carried out in mice according to the method Saganuwan, 2005.

#### *Parasite inoculation*

First, the amount of parasitized red blood cells in a thin blood film were counted, and a Neubauer hemocytometer (JSB348) was used to calculate the % parasitaemia of a mouse previously infected with *P. berghei*. To inoculate the experimental mice, infected blood was collected from the donor mouse and diluted with normal saline. Naïve mice were then inoculated intraperitoneally with 0.2 mL of the infected blood containing $1.0 \times 10^7$ parasitized erythrocytes (day 0). Treatment was withheld for 72 hours (Days 1-3) to allow for the development of infection and was started as soon as blood testing on the mice revealed that they had parasitemia (Ryley and Peters, 1970).

### Experimental Design

The mice were then grouped into seven (7) groups of five (5) mice each. Animals in Group 1 were neither inoculated nor treated with the decoction (normal control). Animals in Group 2 were inoculated with *P. berghei* but not treated (untreated control). The animals in Group 3 were inoculated and treated with 20 mg/kg body weight of chloroquine (treated control). Animals in Groups 4, 5, and 6 were also inoculated and administered 100, 150, and 300 mg/kg body weight of the decoction respectively. Those in Group 7 were not inoculated but received 300 mg/kg body weight of the decoction as well (control). Daily oral treatments were given for four days (Days 4-7). After the completion of treatment, blood from the tail was collected from each mouse in the infected groups on days 8, 9, 10, and 11 post-inoculation; the collected blood was used to make thin films on microscope slides; the films were fixed with methanol, stained with Giemsa stain, and examined under the microscope (Olympus, LMI 406) using the x100 (oil immersion) objective lens to determine the parasitaemia. Percentage chemosupression caused by treatment with the decoction was computed by deducting the average parasitemia in the treated group from the average parasitaemia in the untreated group and expressing the result as a percentage of the untreated group's parasitaemia.

### Blood Sample Collection and Preparation

A day after the completion of treatment, the mice were anaesthetized with diethyl ether, and the blood was collected by cardiac puncture into clean EDTA bottles for analysis of haematological parameters and some in sterile plain containers for liver function tests. The liver tissues were collected after the dissection of the mice and fixed in a 10% formalin solution for histological investigations.

### Hematological Analysis

Haematological parameters were analyzed using an automated hematology analyzer (MON1102, MON Scientific).

### Determination of Liver Function Markers and Indices

The activities of Serum Alanine Aminotransferase (ALT), Serum Alkaline Phosphatase (ALP), and Serum Aspartate Transaminase (AST) were determined according to the method of Reitman and Frankel, 1957. Serum albumin was measured using the method of Doumas *et al.* (1971), bilirubin concentration was measured according to the method of Grant (1987) and serum total protein was measured using the Biuret method as described by Gornall *et al.* (1949).

**Histopathological Analysis**
Changes in the normal architecture of the liver tissues were assessed using the method of Strate *et al.* (2005).

**Data analysis**
Grouped data were calculated using the Statistical Package for Social Sciences (Version 27.0 SPSS-PC Inc. Chicago); these were expressed as mean ± standard error of mean (SEM), and the One-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for post hoc were used to assess the statistical significance of the differences; p-values <0.05 were deemed significant.

## RESULTS AND DISCUSSION

**Results**
Table 1 shows some phytochemicals detected in the decoction; phenolics had the highest concentration.

**Table 1: Some Phytochemicals detected in the Decoction of *Carica Papaya, Psidium Guajava* and *Mangifera Indica* leaves**

| Phytochemicals | Concentration (%) |
|---|---|
| Saponins | 2.16 ± 0.03 |
| Tannins | 9.03 ± 0.01 |
| Alkaloids | 5.71 ± 0.02 |
| Flavonoids | 3.94 ± 0.01 |
| Phenolics | 12.06 ± 0.02 |
| Glycosides | 1.11 ± 0.02 |
| Steroids | 0.67 ± 0.01 |
| Terpenoids | 0.91 ± 0.03 |

Values are Mean ± SEM of 3 replicates

The results of the acute toxicity test of the decoction on mice are shown in Table 2. The calculated $LD_{50}$ was above 5000 mg/kg body weight.

**Table 2: $LD_{50}$ of the Decoction of Psidium Guajava, Carica Papaya and Mangifera Indica leaves in mice**

| Group | Number of Animals | Dose (mg/kg b.wt) | Dose Difference (a) | Deaths | Mean Deaths (b) | Product (a*b) |
|---|---|---|---|---|---|---|
| 1 | 5 | 1000 | 1000 | 0 | 0 | 0 |
| 2 | 5 | 2000 | 1000 | 0 | 0 | 0 |
| 3 | 5 | 3000 | 1000 | 0 | 0 | 0 |
| 4 | 5 | 4000 | 1000 | 0 | 0 | 0 |
| 5 | 5 | 5000 | 1000 | 2 | 0.4 | 400 |
| 6 | 5 | 6000 | 1000 | 5 | 1 | 1000 |
| **Total** | | | | | | **1400** |

$LD_{50}$ = Least dose that killed all the animals - $\frac{Sum\ of\ product}{Number\ of\ animals\ per\ group}$

$LD_{50} = 6000 - \frac{1400}{5}$

$LD_{50}$ = 5720 mg/kg body weight

The effects of the decoction on the parasitaemia of mice infected with P. berghei on days 8 to 11 post-inoculation are displayed in Table 3. In vivo antimalarial studies, drugs/extracts or extract products that elicit less than 30% chemosupression are often considered ineffective; those that elicit between 30% and 50% chemosupression are considered moderately effective, and those that evoke more than 50% chemosupression are deemed effective. Only the group treated with 300 mg/kg body weight of the decoction showed activity on day 5 (above 50% chemosupression) but there was activity on days 6 and 7 for all the doses.

**Table 3: Effects of the Decoction of Carica papaya, Psidium guajava, and Mangifera indica Leaves on parasitaemia of mice infected with Plasmodium berghei (NK-65)**

| | Parasitemia (%) (% Chemo-suppression) | | | |
|---|---|---|---|---|
| **Groups** | **Day 8** | **Day 9** | **Day 10** | **Day 11** |
| **Untreated control** | 31.05 | 35.11 | 39.41 | 43.58 |
| **Chloroquine 20mg/kg b.wt** | 15.22 **(50.98)** | 7.13 **(79.69)** | 4.39 **(87.24)** | 1.05 **(97.59)** |
| **100 mg/kg b.wt** | 27.11 **(12.68)** | 24.29 **(30.81)** | 19.55 **(43.18)** | 15.80 **(63.74)** |
| **150 mg/kg b.wt** | 23.41 **(24.64)** | 20.53 **(41.52)** | 14.61 **(52.31)** | 10.13 **(76.75)** |
| **300 mg/kg b.wt** | 20.57 **(33.75)** | 16.37 **(50.37)** | 12.01 **(65.09)** | 8.39 **(80.97)** |

Values are means of 5 replicates

**Effects of the Decoction of *Carica papaya, Psidium guajava* and *Mangifera indica* Leaves on Liver Function Markers**
The results of the effects of the decoction on liver enzyme and non-enzyme markers of mice are presented in Table 4. Following inoculation with *P. berghei*, the activities of AST, ALT, ALP, and the concentrations of total and direct bilirubin (T.BIL) and D.BIL were significantly elevated ($p < 0.05$), while total protein (TP) and albumin (ALB) were significantly decreased ($p < 0.05$). Administration of the decoction however significantly decreased ($p<0.05$) the activities of AST, ALT, ALP, and concentration of T.BIL and D.BIL and increased the concentrations of TP and ALB with the highest activity observed at 300 mg/kg body weight of the decoction. The decoction control group showed no significant ($p>0.05$) difference in terms of biochemical indices assayed in contrast to normal control.

**Table 4: Effects of the Decoction of Carica papaya, Psidium guajava, and Mangifera indica Leaves on Liver Function Markers**

| Group | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | TP (g/L) | ALB (g/L) | D.BIL (mg/dl) | T.BIL (mg/dl) |
|---|---|---|---|---|---|---|---|
| **Normal control** | 40.57 ± 0.34$^{a}$ | 41.15± 0.21$^{a}$ | 77.86± 0.18$^{a}$ | 57.36± 0.33$^{a}$ | 35.14± 0.03$^{a}$ | 0.87± 0.04$^{a}$ | 3.27 ± 0.13$^{a}$ |
| **Untreated control** | 77.19 ± 0.01$^{b}$ | 69.33± 0.39$^{b}$ | 114.01± 0.03$^{b}$ | 40.34± 0.03 $^{b}$ | 23.10±0.33$^{b}$ | 1.57 ± 0.24$^{b}$ | 6.57± 0.20$^{b}$ |
| **Chloroquine 20mg/kg b.wt** | 39.71 ± 0.13$^{a}$ | 40.42 ± 0.11$^{a}$ | 78.92 ± 0.13$^{a}$ | 55.34 ± 0.15$^{a}$ | 34.31 ±0.13$^{a}$ | 0.84 ± 0.03$^{a}$ | 3.13 ± 0.32$^{a}$ |
| **100 mg/kg b.wt** | 66.17 ± 1.76 $^{c}$ | 58.67 ± 0.90$^{c}$ | 102.00 ± 0.65$^{c}$ | 45.45 ± 0.67$^{c}$ | 28.32 ± 0.21$^{c}$ | 1.39± 0.01$^{c}$ | 5.39± 0.05$^{b}$ |
| **150 mg/kg b.wt** | 53.67 ± 1.24 $^{d}$ | 50.13 ± 0.25$^{d}$ | 89.07 ± 0.53$^{d}$ | 48.23 ± 0.18$^{d}$ | 30.87 ± 0.17 $^{c}$ | 1.14± 0.01$^{d}$ | 3.44± 0.15$^{a}$ |
| **300 mg/kg b.wt** | 42.25 ± 0.55 $^{a}$ | 42.32 ± 2.66 $^{a}$ | 79.02 ± 0.611$^{a}$ | 52.72± 0.51$^{e}$ | 33.67 ± 0.25$^{d}$ | 0.89± 0.01$^{a}$ | 3.28± 0.09$^{a}$ |
| **300 mg/kg b.wt (Decoction Control)** | 39.33±0.97$^{a}$ | 41.01±2.08$^{a}$ | 76.67 ± 0.63$^{a}$ | 56.91± 0.11$^{a}$ | 37.19± 0.40$^{a}$ | 0.85±0.03$^{a}$ | 3.18±0.01$^{a}$ |

The data is presented as the Mean± standard error of mean (SEM) based on five replicates. Significant differences ($p < 0.05$) exist between numbers in the same column with different superscripts.

Table 5 presents the results of the effects of the decoction on haematological parameters. Infection with Plasmodium resulted in a notable decrease ($p < 0.05$) in RBC, Hb, PCV and platelets and a substantial decline in the white blood cell count (WBC). These changes were improved towards normal by treatment. The decoction didn't produce any notable difference ($p > 0.05$) in normal mice as shown by the findings in the decoction-only group.

**Table 5: Effects of the Decoction of Carica papaya, Psidium guajava, and Mangifera indica leaves on Haematological parameters of mice inoculated with Plasmodium berghei (NK-65)**

| Group | RBC ($\times10^{12}$/L) | HB (g/dl) | WBC ($\times10^{9}$/L) | PCV (%) | PLT ($\times10^{9}$/L) |
|---|---|---|---|---|---|
| **Normal control** | $7.34 \pm 0.03^{a}$ | $19.22 \pm 0.18^{a}$ | $12.48 \pm 0.11^{a}$ | $41.82 \pm 0.16^{a}$ | $971.02 \pm 0.16^{a}$ |
| **Untreated control** | $4.01 \pm 0.10^{b}$ | $9.92 \pm 0.21^{b}$ | $29.46 \pm 0.18^{b}$ | $27.36 \pm 0.22^{b}$ | $764.60 \pm 0.25^{b}$ |
| **Chloroquine 20mg/kg b.wt)** | $7.40 \pm 0.17^{a}$ | $18.76 \pm 0.07^{a}$ | $13.60 \pm 0.15^{a}$ | $39.06 \pm 0.12^{a}$ | $968.20 \pm 0.19^{a}$ |
| **100 mg/kg b.wt** | $4.38 \pm 0.05^{b}$ | $11.02 \pm 0.15^{c}$ | $25.80 \pm 0.13^{c}$ | $30.36 \pm 0.25^{c}$ | $790.40 \pm 0.07^{c}$ |
| **150 mg/kg b.wt** | $4.65 \pm 0.05^{b}$ | $13.18 \pm 0.10^{c}$ | $19.02 \pm 0.23^{d}$ | $33.14 \pm 0.1^{c}$ | $801.08 \pm 0.18^{d}$ |
| **300 mg/kg b.wt** | $7.46 \pm 0.04^{a}$ | $18.28 \pm 0.19^{a}$ | $13.34 \pm 0.08^{a}$ | $39.14 \pm 0.19^{a}$ | $954.20 \pm 0.09^{a}$ |
| **300mg/kg (Decoction Control)** | $7.30 \pm 0.28^{a}$ | $19.44 \pm 0.33^{a}$ | $12.16 \pm 0.34^{a}$ | $41.18 \pm 0.14^{a}$ | $969.80 \pm 0.10^{a}$ |

The data is presented as the Mean ± standard error of mean (SEM) based on five replicates. Significant differences ($p < 0.05$) exist between values in the same column with different superscripts.

**Effects of the Decoction of *Carica papaya, Psidium guajava and Mangifera indica* Leaves on liver Histopathology of Mice**

Microscopic examination of the tissue sections of liver of mice showed distorted architecture with noticeable differences in the histological and cellular structures of the of the hepatocytes, central vein and portal artery of the untreated control group compared to the normal control. Treatment with leaf decoction however restored tissue architecture towards normal in a dose dependent manner (Plates 1 and 2).

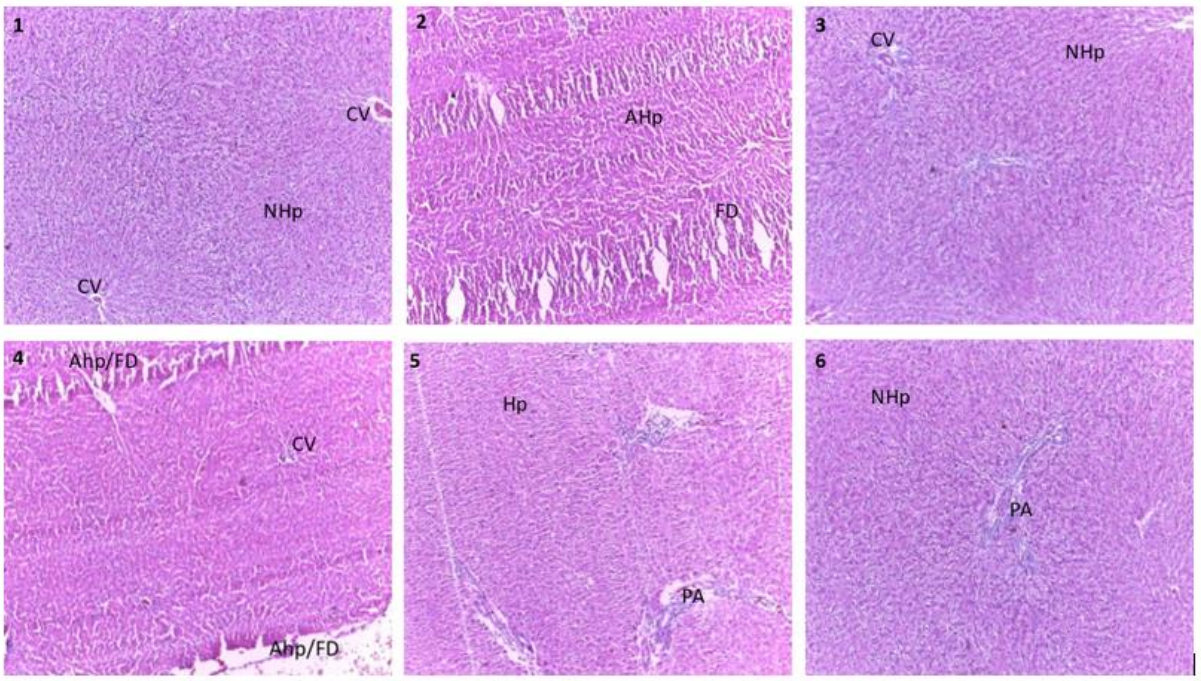


Plate 1: Photomicrographs of the liver tissue of mice infected with *P. berghei* and administered various doses of the decoction of *C. papaya, P. guajava, and M. indica* leaves for 4 days. Groups 1, 2, 3, 4, 5, and 6: Normal control, untreated control, chloroquine 20 mg/kg body weight, 100, 200 and 300 mg/kg body weight of the decoction respectively (H and E x 400). Hp/Nhp =normal hepatocytes, Ahp=abnormal hepatocytes, FD= fatty degeneration, PA= portal artery, CV=central vein

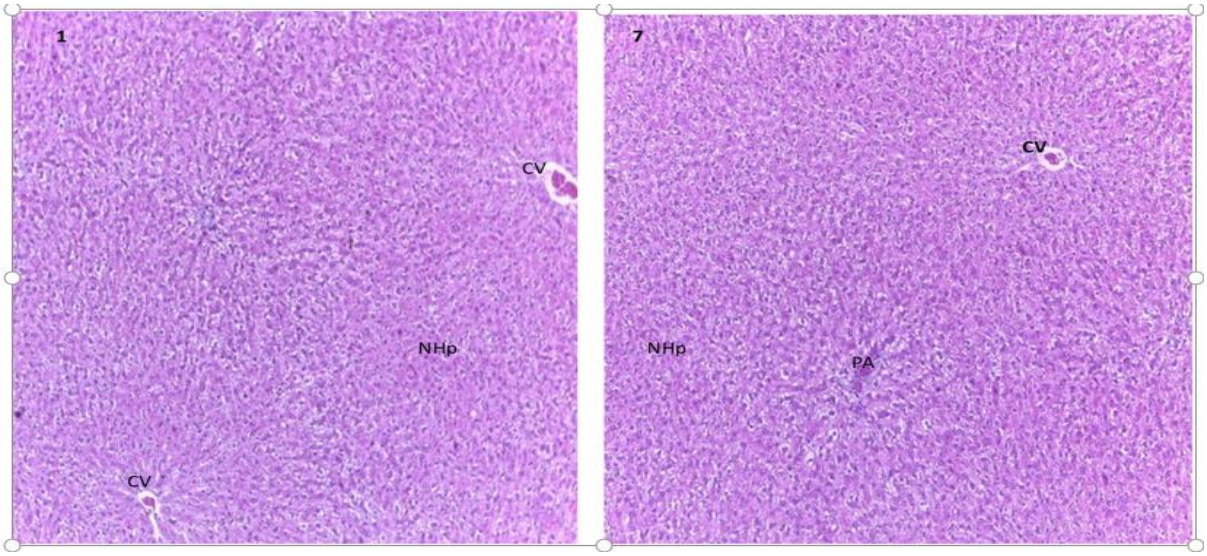


Plate 2: Photomicrographs of the liver tissue of mice administered various doses of the decoction of *C. papaya, P. guajava and M. indica* leaves for 4 days. Groups1 and 7: Normal control (1) and Decoction control (7) (H and E x 400)

**Discussion**

Plants have been a major source of new compounds with medicinal properties and are thus, widely used in the management of several ailments worldwide. This is especially true for the traditional management of malaria in sub-Saharan Africa where it is endemic and continues to be a significant health burden with severe consequences. The impact of novel therapeutic compounds from plants is illustrated by two of the most significant antimalarial medications used in the treatment of malaria, quinine and artemisinin. These were originally obtained from the plants *Cinchona officinalis* and *Artemisia annua*, respectively (Mohammadi *et al.,* 2020).

The therapeutic efficacy of plants is because of certain phytochemical compounds they contain most of which are secondary metabolites. Plants normally use these secondary metabolites to ward off amoebae, bacteria, fungi, insects, large animals, and potential predators and also compete with other plants (Dixon *et al.,* 2024). However, when properly harnessed, these secondary metabolites can be exploited for their therapeutic value. In this study, the decoction of *Carica papaya*, *Psidium guajava*, and *Mangifera indica* leaves was found to contain saponins, tannins, alkaloids, flavonoids, phenolics, glycosides, steroids, and terpenoids in various amounts (Table 1). Some of these compounds have been shown to be effective against malaria either singly on their own or have a synergistic effect in combination with other phytoconstituents (Igbokwe *et al.,* 2021; Ounjaijean and Sosak, 2023). These phytochemicals have also been reported to be the major constituents of plants with antimalarial activities (Anamul *et al.,* 2022; Pandey *et al.,* 2023). The decoction of *Carica papaya*, *Psidium guajava*, and *Mangifera indica* leaves exhibited antimalarial efficacy in a dose-dependent manner similar to that of the orthodox drug, chloroquine (Table 3). The antimalarial activity observed could be attributed to the synergistic effects of the phytoconstituents found in the decoction (Kaur *et al.,* 2023; Saufi *et al.,* 2023). Some of the phytochemicals identified in this study have been reported to kill parasites both *in vivo* and *in vitro* using one or more mechanisms. Phenolic compounds are a significant class of secondary metabolites that function as principal antioxidants or scavengers of free radicals (Pal, 2023). It has also been reported that the mechanism of their antimalarial efficacy is by inhibiting the parasite's protein synthesis, elevating the red blood cell oxidation and counteracting the oxidative damage caused by the malaria parasite while some inhibit the polymerization of haem to the malarial pigment haemozoin by inhibiting β-haematin formation (Builders *et al*., 2010; Bassey *et al.,* 2022). Terpenes act by inhibiting parasite growth and nutrient intake by inhibiting the permeation pathway while flavonoids exert their activity by inhibiting the uptake of essential amino acids into infected red blood cells. Alkaloids on the other hand disrupt parasite protein metabolism as well as inhibit some enzymes (Kaur and Aurora, 2015; Tajuddeen and Van Heerden, 2019).

Previous studies have documented the antimalarial activity of the individual leaves, and it shows that findings from this investigation with the combination of the leaves (decoction) significantly improved the changes caused by infection with malaria (Amat-ur-Rasool *et al*., 2020; Jiang *et al*., 2020; Nafiu *et al.,* 2021). This may be because decoctions mimic multidrug strategies in therapeutic applications which can increase efficacy, improve outcomes, reduce the length of treatment and lower the chance of recurrence (Nguyen *et al.,* 2023).

The liver has several physiological roles most notably in integrating systemic metabolism for glucose and fatty acid metabolism. During malaria infection, the interaction between the host and parasite also takes place in the liver (sporozoite incubation stage) and the activities of the growing parasite in the liver disrupt its functions which manifests as changes in the activities of some enzymes in the liver especially ALT and AST as well as ALP (Zailani *et al.,* 2020; Hu *et al.,* 2023). Parasite activities in the liver also lead to hepatomegaly, enhanced elimination of both infected and uninfected red blood cells by the spleen leading to a reduction in red blood cell count, hyperbilirubinaemia, and jaundice (Arfijanto and Wahyudi, 2023). The result of this study agrees with these reports as evidenced by the notable decline in the RBC count, haemoglobin, packed cell volume, platelets, total protein, albumin and a significant rise in the activities of ALP, ALT and AST, and bilirubin concentration because of infection with *P. berghei* (Tables 4 and 5). The host liver is affected in the early stage of infection with malaria parasites because the parasite incubates in the liver before the erythrocytic stage. Sporozoite invasion of the liver causes injury to the hepatocytes because hepatic blood flow is impeded by parasitized red blood cells adhering to endothelial cells, which

blocks the sinusoids and prevents blood from entering the liver. This leads to the leakage of the liver enzymes into the bloodstream. This phenomenon may be the cause of elevated activities of these enzymes in the serum as observed in this study (Formaglio *et al.,* 2023). There might also be associated hepatocyte necrosis and bile stasis because bilirubin transport is compromised, and enhanced immune-mediated hemolysis of non-parasitized and parasitized red blood cells which causes jaundice and subsequently affect function parameters and enzyme activities within the liver (Enechi *et al.,* 2021). In this experiment, parasite infection resulted in a significant increase in all the liver function indices aside from serum total protein and albumin which notably reduced. Invasion of hepatocyte by malarial parasites can lead to inflammatory reactions linked to parasite metabolic by-products. This hepatocyte membrane disturbance leads to the leakage of intracellular cytosolic enzymes elevating their activities in the serum as seen in this study (Table 4) (Orabueze *et al*., 2020). Similarly, the increased concentrations of serum bilirubin in the untreated group as opposed to the control could be because of increased hemoglobin destruction by the malaria parasite since bilirubin is a byproduct of hemoglobin which is usually broken down in the liver. The endocytic consumption of haemoglobin by the parasites within the red blood cells and subsequent production and local accumulation of haemozoin produced by the parasite throughout the blood stage of its life cycle leads to increased haemolysis and the elevation of bilirubin concentration (Onyamboko *et al.,* 2023). Its accumulation in serum in this study suggests dysfunction of hepatic cells because of infection. Treatment with the leaf decoction of *Psidium guajava, Carica papaya,* and *Mangifera indica* restored the activities of these indices towards normal as the dose increased with the highest activity seen at 300 mg/kg body weight of decoction (Table 4). Moreover, the changes in the normal architecture of the liver tissue caused by infection were improved by treatment with the decoction (Plate 1). The improvement of these indices after treatment could be because of direct parasite clearance by components of the decoction (Table 3) and the possible ability of the decoction to improve hepatic status in malaria by improving the affected liver cells of parasitized mice. Liver injury that resulted from malaria has also been associated with oxidative stress, indicating that the hepatocyte modulation by the decoction in this study could be associated with some of its secondary metabolites that have antioxidant activities (Table 1).

One of the most frequent consequences of malaria is changes in haematological indices; having a huge impact on the pathogenesis of the disease and leading to changes in blood and blood cell counts. This occurs during the asexual stage of the parasite's life cycle in the erythrocytes resulting in biochemical alterations in both the morphological and numerical changes of all the blood cell indices. When erythrocytes become parasitized, the parasites release chemicals on their surface that cause vascular adhesion as well as the adherence of red blood cells (parasitized or otherwise), thus boosting the breakdown of the red blood cells by splenic clearance; additionally, malaria induces dyserythropoiesis thus distorting the erythropoietic balance (Milner *et al*., 2020). The parasite spends this part of its life cycle in the erythrocyte digesting and ingesting its haemoglobin leading to malarial anaemia, thrombocytopenia, and leucocytosis; although these changes depend on the parasite species, severity of illness, and the immune status of the host, they mostly correlate well with the degree of parasitaemia (Antwi-Baffour *et al.,* 2023). Some of the changes in haematological parameters because of infection were observed in this study because infected mice had significantly reduced red blood cell count, packed cell volume and platelets, haemoglobin, and an elevated level of white blood cells (Table 5). The reduction in the concentration of these parameters could be attributed to the parasitic infiltration of the red blood cells, the use of haemoglobin as a food supply during the blood stage of the life cycle of the parasite and the physical destruction of red blood cells infiltrated by the parasite. Administration of the decoction of *Carica papaya, Psidium guajava,* and *Mangifera indica* however improved these parameters towards normal as the parasitaemia count decreased.

The most reliable method for assessing pathological alterations in tissues and organs caused by treatment is through histopathological examination (OECD, 1995). The untreated control group showed alterations in the normal liver tissue architecture which are indicative of injury to the liver caused by malaria. Treatment with the leaf decoction however was able to reduce parasitaemia and ameliorate the effects of *P. berghei* infection on the liver tissue; this effect increased positively with increased doses of the decoction (Plate 1).

All the findings related to the decoction control group showed that treatment with the decoction alone did not substantially change any of the parameters evaluated (Tables 4 and 5) and it did not change the typical anatomy of the liver tissue (Plate 2). This, in addition to the calculated oral $LD_{50}$ being above 5000 mg/kg body weight (Table 2) implies that the leaf decoction of *Psidium guajava, Carica papaya,* and *Mangifera indica* at the doses studied are virtually nontoxic when taken orally because substances or medications are said to be minimally toxic if their oral $LD_{50}$ is above 1000 mg/kg body weight (Elekofehinti *et al*., 2021). Prior research has revealed the $LD_{50}$ of each individual components of the decoction; Timothy *et al*. (2022) found the $LD_{50}$ of *Carica papaya* leaf to be above 5000 mg/kg body weight, Manekeng *et al*. (2019) reported that of *Psidium guajava* to be above 5000 mg/kg body weight while Reddeman *et al*. (2019) reported the $LD_{50}$ of *Mangifera indica* leaves to be above 5000 mg/kg body weight.

## CONCLUSION

The current findings show that the decoction of *Psidium guajava, Mangifera indica* and *Carica papaya* ameliorated the effects of infection with *P.* berghei and this effect intensified as the dose of decoction increased. This observation is possibly due to the synergistic effect of the phytochemical constituents of the decoction.

## REFERENCES

Amat-ur-Rasool, H., Symes, F., Tooth, D., Schaffert, L.N., Elmorsy, E., Ahmed, M., *et al.* (2020). Potential nutraceutical properties of leaves from several commonly cultivated plants. *Biomolecules,* 10: 1556.

Amin, A.H., Bughdadi, M.A., Abo-Zaid, A.H., Ismail, S.A., El-Agamy, A., Alqahtani, H.I.H. (2019). Immunomodulatory effect of papaya (*Carica papaya*) pulp and seed extracts as a potential natural treatment for bacterial stress. *J. Food Biochem,* 43 (12) 13050.

Anamul, H., Khoshnur, J., Tohmina, B., Rownak, J., Shahadat, H., Pereira, D., Veeranoot, N., Christophe, W., Mohammed, R. (2022). Can antimalarial phytochemicals be a possible cure for Covid-19? Molecular docking studies of some phytochemicals to SARS-CoV-2 3C-like protease. *Infect. Disord. Drug Targets*, 22 (1):96-106.

Antwi-Baffour, S., Mensah, B. T. and Johnson, G. (2023). Haematological parameters and their correlation with the degree of malaria parasitaemia among outpatients attending a polyclinic. *Malar. J*, 22, 281. .

Arfijanto, M. V. and Wahyudi, M. I. (2023). Rare case of hyperbilirubinemia due to malarial hepatopathy in severe *falciparum* malaria. *The Indonesian Journal of Gastroentorology, Hepatology, and Digestive Endoscopy*, 24 (2): 172-175.

Arrey, T.P., Franzoi, K.D., Lee, S., Lee, E., Vivarelli, D. (2014). *In-vitro* antiplasmodial activities and synergistic combinations of different solvent extract of the polyherbal product, Nefang. *Bio Med Res Int.* 835013 | https://doi.org/10.1155/2014/835013

Bassey, A A., Samuel, O. O, Ekemini, V. E., Mfonobong, E. U., Anthony, C. P., Johnson, E. and Eseyin, O. (2022). Molecular docking and antimalarial evaluation of natural phenolics and their derivatives on *Plasmodium falciparum* lactate dehydrogenase as schizontocidal drug candidates. *Nig. J Pharma App. Sci. Res*. 11(2):19-26.

Builders, M. I., Wannang, N. N., Ajoku, G. A., Builders, P. F., Orisadipe, A. and Aguiyi, J.C. (2010). Evaluation of the antimalarial potential of *Vernonia ambigua Kotschy* and Peyr (Asteraceae). *Int. J. Pharmacol*. 18(11):1-9.

Chaniad, P., Phuwajaroanpong, A., Plirat, W., Konyanee, A., Septama, A.W., Punsawad, C. (2024). Assessment of antimalarial activity of crude extract of Chan-Ta-Lee-La and Pra-Sa-Chan-Dang formulations and their plant ingredients for new drug candidates of malaria treatment: *In vitro* and *in vivo* experiments. PLoS ONE 19(1): e0296756. https://doi.org/10.1371/journal.pone.0296756 .

Ćujić, N., Katarina, Š., Teodora, J., Dejan, P., Gordana, Z., Svetlana, I. (2016). Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chem*. 194:135-142.

Dixon, R. A., Alexandra, J. and Dickinson, A. (2024). A century of studying plant secondary plant secondary metabolism – from "what" to "where, how, and why?" Plant Physiology, kiad596. http://doi.org/10.1093/plphys/kiad596 .

Doumas, B.T., Watson, W.A. and Biggs, H.G. (1971). Albumin Standard and Measurement of Serum Albumin with Bromocresol Green. *Clin. Chim Acta*. 31, 87-96.

Elekofehinti, O.O., Iwaloye, O., Olawale, F. and Ariyo, E.O. (2021). Saponins in cancer treatment: current progress and prospects. *Pathophysiology*. 28(2): 250–272. https://doi.org/10.3390/pathophysiology28020017.

Enechi, O. C., Okagu, I. U., Amah, C. C., Ononiwu, P. C., Igwe, J. F. and Onyekaozulu, C. R. (2021). Flavonoid-rich extract of *Buchholzia coriacea* Engl. Seeds reverses *Plasmodium berghei*-modified haematological and biochemical status in mice. *Scientific African* 12, e00748. https://doi.org/10.1016/j.sciaf.2021.e00748 .

Eziuche, A., Ugbogua, E., Dikea, M., Ebubechi, U., Lotanna, R., Etumnua, B. (2023). Ethnomedicinal uses, nutritional composition, phytochemistry and potential health benefits of Carica papaya. *Pharm. Res - Modern Chinese Med*. **7**: 100266.

Fidock, D.A., Rosenthal, P.J., Croft, S.L., Brun, R. and Nwaka, S. (2004). Antimalaria drug discovery: efficacy models for compound screening. *Nat. Rev. Drug Discov*, 3(6), 509-520.

Formaglio, P., Wosniak, M. E., Tromer, R. M. (2023). *Plasmodium* sporozoite search strategy to locate hotspots of blood vessel invasion. *Nat. Commun*. 14, 2965. http://doi.org/10.1038/s41467-023-38706-z .

Ghaffarilaleh, V., Fisher, D., Henkel, R. (2019). *Carica papaya* seed extract slows human sperm. *J. Ethnopharmacol*. 241 111972.

Gornall, A. G., Bardawill, C. J. and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J Biol Chem*. 177(2):751-66. PMID: 18110453.

Grant, G.H. (1987). Amino Acids and Proteins. In: *Fundamentals of Clinical Chemistry*, Tietz NW, 3rd edition, Philadephia, USA: WB Saunders Company pp. 328-329.

Hamilton, W.L., Amato, R., Vander Pluijm, R.W., Jacob, C.G., Quang, H.H., ThuyNhien, N.T. (2019). Evolution and expansion of multidrug-resistant malaria in southeast Asia: a genomic epidemiology study. *Lancet Infect.* 19: 943–951.

Hu, X., Zhao, J. and Zhao, J. (2023). Genome-wide liver transcriptomic profiling of a malaria mouse model reveals disturbed immune and metabolic responses. *Parasites Vectors*, 16, 40. http://doi.org/10.1186/s13071-023-05672-w

Igbokwe, U. V., Eze, E. D., Adams, M. D., Rabiu, K. M., Ezekiel, I., Ajeka, P. O., & Okpara P. O. (2021). Anti-malarial effects of five traditional Nigerian medicinal plant extracts on *Plasmodium berghei*-infected rats. *FUDMA Journal of Sciences*, *5*(2), 7 - 17. https://doi.org/10.33003/fjs-2021-0502-46

Jiang, L., Lu, J., Qin, Y., Jiang, W., Wang, Y. (2020). Antitumor effect of guava leaves on lung cancer: A network pharmacology study. *Arab. J. Chem.* **13**: 7773–7797.

Kaur, R. and Arora, S. (2015). Alkaloids—Important Therapeutic Secondary Metabolites of Plant Origin. *J. Crit. Rev.* 2:1–8.

Kaur, H., Kaur, H., Srivastava, S. (2023). The beneficial roles of trace and ultra trace elements in plants. *Plant Growth Regul*. 100, 219-236. http://doi.org/10.1007/s10725-022-00837-6.

Kaur, H., Singh, A., Mukhtar, H. M., Singh, H. (2023). Hybrid alkaloids- an approach towards development of better antimalarial therapeutics. *Studies in Natural Product Chemistry*, 76:199-245.

Manekeng, H. T., Mbaveng, A. T., Ntyam Mendo, S. A., Agokeng, A. D. and Kuete, V. (2019). Evaluation of Acute and Subacute Toxicities of *Psidium guajava* Methanolic Bark Extract: A Botanical with *In Vitro* Antiproliferative Potential. *Evidence-based Complementary and Alternative Medicine (eCAM)*, 8306986.

Manoj, K., Vivek, S., Maharishi, T., Muzaffar, H., Sushil, C., Minnu, S., *et al.* (2021). Mango (*Mangifera indica L.)* Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. *Antioxidant*, 10: 299.

Milner, E.M., Kariger, P., Pickering, A.J., Stewart, C.P., Byrd, K. and Lin, A. (2020). Association between malaria infection and early childhood development mediated by anemia in rural Kenya. *Int. J Environ Res Public Health*, 17(3):902.

Mohammadi, S., Jafari, B., Asgharian, P., Martorell, M. and Shariff-Rad, J. (2020). Medicinal plants used in the treatment of malaria: A key emphasis to *Artemisia*, *Cinchona*, *Cryptolepis*, and *Tabebuia genera*. *Phytother. Res*, 34(7):1556-1569.

Nafiu, M., Adeyinka, A., Taoheed, A. and Anofi, A. (2021). Antimalarial activity and biochemical effects of saponin-rich extract of Dianthus basuticus burtt davy in *Plasmodium berghei*-infected mice. *Adv Trad Med*, 1-11.

Naseer, S., Shabbir, H., Naureen, N., Muhammad, P., Madiha, R. (2018). The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clin. Phyto Sci*, 4(1)1-8.

Nguyen, T. D., Gao, B., Amaratunga, C. (2023). Preventing antimalarial drug resistance with triple artemisin-based combination therapies. *Nat. Commun* 14, 4568. http://doi.org/10.1038/s41467-023-39914-3.

Oche, O., Nathan, H., Joseph, I., Vincent, A.U., Stanley, I.R.O. and Omiagocho, T.I. (2016). Antimalarial potential of *Carica papaya* and *Vernonia amygdalina* in mice infected with *Plasmodium berghei*. *J Trop Med*; 8738972.

OECD (1995). OECD Guideline for Testing of Chemicals. Repeated Dose 28-Day Oral Toxicity Study in Rodents; Organisation for Economic Co-operation and Development: Paris, France, pp 425.

Orabueze, C. I., Obi, E., Adesegun, S. A. and Coker, H. A. (2020). Potential Antimalarial activity of *Coccina barteri* leaf extract and solvent fractions against *Plasmodium berghei* infected mice. *J Ethnopharmacol*, 248, 112334, https://doi.org/10.1016/j.jep.2019.112334.

Ounjaijean, S. and Somsak, V. (2023). Synergistic antimalrial treatment of *Plasmodium berghei* infection in mice with dihydroartemisinin and *Gymnema inordorum* leaf extract. *BMC Complimentary Med*. Ther. 23:20.

Onyamboko, M. A., Olupot-Olupot, P., Were, W. (2023). Factors affecting haemoglobin dynamics in African children with acute uncomplicated *Plasmodium falciparum* malaria treated with a single low dose primaquine or placebo. *BMC Med* 21, 397. http://doi.org/10.1186/s12916-023-03105-0 .

Pal, C. (2023). Redox modulating small molecules having antimalarial efficacy. *Biochem. Pharmacol*, 218, 115927.
Pandey, S. K., Anand, U., Siddiqui, W. A., Tripathi, R. (2023). Drug development strategies for malaria with the hope for new antimalarial drug discovery-An update. *Adv. Med*, http://doi.org/10.1155/2023/5060665.

Reddeman, R. A., Glávits, R., Endres, J. R., Clewell, A. E., Hirka, G., Vértesi, A., Béres, E. and Szakonyiné, I. P. (2019). A Toxicological Evaluation of Mango Leaf Extract (*Mangifera indica*) Containing 60% Mangiferin. *J Toxicol*, 4763015.

Reitman, S. and Frankel, S. (1957). Glutamic – pyruvate transaminase assay by colorimetric method. *Am. J Clin. Path*, 28:56.

Rocklov, J. and Dubrow, R. (2020). Climate change: an enduring challenge for vector-borne disease prevention and control. *Nat. Immunol*. 21: 479–483.

Ryley, J. F. and Peters, W. (1970). The antimalarial activity of some quinolone esters. *Ann. Trop. Med Parasitol*, 64,209-222. http://doi.org/10.1080/00034983.1970.11686683.

Sadeghi, S., Valadkhani, Z., Tafreshi, A.S., Arjmand, M., Nahravanian, H., Vahabi, F., Soori, N., Mohammadi, M., Meigooni, M. and Zamani, Z. (2020). A Study of Synergy of Combination of Eosin B with Chloroquine, Artemisinin, and Sulphadoxine-Pyrimethamine on *Plasmodium falciparum In Vitro* and *Plasmodium berghei In Vivo*. *J Trop Med.* 1;2020:3013701 http://doi.org/10.1155/2020/3013701 .

Saganuwan, S. A. (2005). Arithmetic-Geometric-Harmonic (AGH) method of rough estimation of median lethal dose ($LD_{50}$) using up- and- down procedure, *J Drug Metab Toxicol*, 6 (2) 180.

Santana, L.F., Inada, A.C., Espirito, B.L.S.D., Santo, F., Pott, F.M., Alves, R.C.A. (2019). Nutraceutical potential of *Carica papaya* in metabolic syndrome. *Nutrients* 11(7):1608.

Saufi, N. A., Hatta, U. M., Rashid, F. A., Mohammat, M. F. (2023). Alkaloids as antimalarial compounds: A review of recent studies. *Mini Rev Org Chem*, 20(8):786-799.

Singh, S.P., Kumar, S., Mathan, S.V., Tomar, M.S., Singh, R.K., Verma, P.K. (2020). Therapeutic application of *Carica papaya* leaf extract in the management of human diseases. *Daru* 28 (2) 735–744.

Sinka, M.E., Pironon, S., Massey, N.C., Longbottom, J., Hemingway, J., Moyes, C.L. (2020). A new malaria vector in Africa: predicting the expansion range of *Anopheles stephensi* and identifying the urban populations at risk. *Proc. Nat. Acad. Sci.* 117: 24900–24908.

Strate, T., Mann, O. and Kleinhans, H. (2005). Microcirculatory function and tissue damage is improved after therapeutic injection of bovine hemoglobin in severe acute rodent pancreatitis. Pancreas. 3(30): 254–259.

Suh, P.F., Elanga-Ndille, E., Tchouakui, M., Sandeu, M.M., Tagne, D., Wondji, C. (2023). Impact of insecticide resistance on malaria vector competence: a literature review. *Malaria J.* 22, 1–11.

Tajuddeen, N. and Van Heerden, F. G. (2019). Antiplasmodial natural products. *Malar J.* 18:404 https://doi.org/10.1186/s12936-019-3026-1 .

Trease, G. E. and Evans, W.C. (1989). *Pharmacognosy*. 11th Edition, Bailliere Tindall, London: Pp. 45-50.

Uzor, P. F. Ishiwu, B. U., and Nwodo, N. J. (2020) *In vivo* antimalarial effect of *Ananas comosus (L)* Merr

(*Bromeliaceae*) fruit peel, and gas chromatography-mass spectroscopy profiling: a possible role for polyunsaturated fatty acid, *J Trop. Pharm Res*, 19, (1) 137–145.

WHO, (2022). World Malaria Report 2022. World Health Organization. Geneva Switzerland, ISBN-9789241565158.

WHO, (2020). World Malaria Report 2020. World Health Organization. Geneva Switzerland, ISBN-9789245565158.

Zailani, A. H., Iliyas, M. B., Benjamin, l., Ibrahim, B. A., Ubah, B. and Lamiya, A. (2020). *Cola nitida* leaf phytochemicals improve liver function indices of mice infected with *Plasmodium berghei* (NK-65). *J Med. Plants studies*, 8(2):143.