



Curative antimalarial effects of combined aqueous leaf extracts of *Carica papaya*, *Cymbopogon citratus*, and *Artemisia annua* on mice infected with *Plasmodium berghei*.

Abstract: The study evaluated the antimalarial effects of combined aqueous extracts of *Carica papaya*, *Cymbopogon citratus*, and *Artemisia annua* on mice infected with *Plasmodium berghei*. Fresh leaves of *C. papaya*, *C. citratus*, and *A. annua* were authenticated and pulverised. Aqueous extracts of each plant were qualitatively and quantitatively analysed for phytochemicals. Acute toxicity of the extracts was also assessed using Lorke's method. Forty-eight mice were randomised into 8 groups of 6 mice each (n=6) and treated orally with extracts for seven days. The next day, blood samples were analysed for parasitemia counts and biochemical parameters. The mice's body weight, temperature, malondialdehyde (MDA) and superoxide dismutase (SOD) were also assessed. Results show phytochemicals were present in the extracts in varying amounts. The combined extract's LD₅₀ was 3,807.89 mg/kg, with no mortality at tested doses. The extracts showed no significant (p > 0.05) changes in liver function parameters. No significant differences (p > 0.05) in MDA and SOD levels in treated versus control groups overall. Moreover, all extracts had a dose-dependent statistically significant (p < 0.05) percentage reduction of plasmodium in this order: 380.79 mg/kg of *C. citratus* (63.48%), 190.40 mg/kg of *A. annua* (61.40%), 380.79 mg/kg of *C. papaya* (60.88%), and 190.40 mg/kg of combined extract (58.27%). The study established that combined aqueous extracts of the three plants have significant antimalarial activities and negligible toxicities, supporting their application in herbal medicine.

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1.Introduction

Malaria is a life-threatening disease caused by *Plasmodium* parasites that are transmitted to humans through the bites of infected female *Anopheles* mosquitoes [1]. Symptoms typically manifest 10-15 days following the infective mosquito bite in a non-immune individual [2]. When severe, malaria becomes a medical emergency. Children and pregnant women are



always at risk when compared to healthy adults and non-pregnant women. Severe malaria in children often causes severe anaemia, metabolic acidosis-related respiratory distress, or cerebral malaria [3]. Moreover, foetal complications, maternal morbidity, and mortality can result from severe malaria during pregnancy [4]. The vector-borne infectious disease malaria is still a major problem for public health around the world, especially in tropical areas like Nigeria.

In Nigeria, malaria is a significant public health issue, resulting in approximately 68 million cases and 194,000 fatalities in 2021 [5]. Malaria, an acute, life-threatening parasitic infection transmitted by the *Anopheles* mosquito, represents a substantial global health concern [6]. According to the World Malaria Report 2023, malaria is the most negatively impacting vector-borne illness globally [7]. Nigeria bears the greatest load of malaria on a worldwide scale, representing approximately 27% of the total global burden. According to WHO [8], the severe manifestation of malaria has caused 194,000 fatalities in 2021, with over 80% of them occurring in children below the age of 5. This amount constitutes 31% of the total number of malaria-related fatalities worldwide, with 40% occurring in the WHO African Region [8].

Despite advancements in malaria prevention and treatment, which have spurred the development of anti-malaria vaccines, resistance has remained the most formidable obstacle. Reinvestigating traditional remedies is one strategy for the development of innovative antimalarial treatments [9]. With developing resistance to artemisinin-based combination treatments, it is critical to accelerate the research and development of novel antimalarial medicines. Herbal remedies are essential in the development of novel pharmaceuticals. Herbal medicine is now widely used in communities to treat malaria symptoms as an alternative to conventional antimalarial drugs. However, the efficacy and safety of the majority of herbal remedies have

yet to be confirmed [10]. This study investigated the antimalarial effects of combined aqueous extracts of *Carica papaya*, *Cymbopogon citratus*, and *Artemisia annua* on mice infected with *Plasmodium berghei*.

2. Materials and Methods

Collection of Plants and Identification

Fresh leaves of *Carica papaya*, *Cymbopogon citratus*, and *Artemisia annua* were collected from the Faculty of Pharmaceutical Sciences at Nnamdi Azikiwe University, Agulu Campus. A taxonomist authenticated these plants, with voucher numbers issued for each: *Cymbopogon citratus* (NAUH-187A), *Artemisia annua* (NAUH-258A), and *Carica papaya* (NAUH-190A) at the Department of Pharmacognosy and Traditional Medicine.

Plasmodium parasite

Plasmodium berghei (NK65) was obtained from the Nigerian Institute of Medical Research (NIMR) in Yaba, Lagos, Nigeria. This institute is a vital resource for acquiring *Plasmodium berghei* strains for study in Nigeria. NIMR supplied infected donor mice from which the parasite was obtained.

Animals Selection

Healthy adult albino mice of average weight (20–25 g) were obtained from the Faculty of Veterinary Medicine, University of Nigeria. They were housed in clean metabolic cages with access to clean drinking water and pelleted feed. The study adhered to the National Institute of Health Guidelines for laboratory animals. The mice were randomly selected, marked for identification, and acclimatized for two weeks prior to dosing. The experimental room was maintained at approximately 22°C, with humidity between 30% and 70%. Lighting followed a 12-hour



cycle of light and dark, and deionised water was provided ad libitum.

Extraction of extract from plant leaves

Extraction was carried out by maceration of dried and pulverised plant material as described by Erhirhie and Ilodigwe [11]. For aqueous extract preparation, a total of 35 g of *Carica papaya* (sample A), 70 g of *Cymbopogon citratus* (sample B), 14.5 g of *Artemisia annua* (sample C), and 4 g, 6 g, and 10 g of samples A, B, and C, respectively (sample D), were cold-macerated in 245 ml, 490 ml, 145 ml, and 100 ml of distilled water, respectively. Macerated samples were kept at room temperature for 48 hrs with continuous agitation. At the end of 72 hours, the mixture was filtered using filter paper, and the filtrate recovered was concentrated to a paste-like form using a water bath at 50°C. The percentage yield of the extract was determined using the formula:

$$\text{Percentage yield} = \frac{\text{Weight (g) of concentrated extract}}{\text{Weight (g) of powdered plant material}} \times \frac{100}{1} S$$

Qualitative Phytochemical Screening

The entire aqueous extracts and various fractions were subjected to phytochemical analysis using standard test procedures by Trease and Evans [12], Harbone [13]. Samples were dissolved in distilled water. A volume of 1 ml of each sample was used for each test described below. A 1 ml sample served as a control for comparing each test carried out.

Quantitative Phytochemical Screening

Determination of total tannin content was carried out using the Folin-Ciocalteu colorimetric method as used by Adegbusi et al. [14], while total alkaloid content was quantified using a procedure similar to that of Prajapati et al. [15]. The total phenolic content was also determined with the Folin-Ciocalteu reagent

method as described by Yadav and Agarwala [16]. Total flavonoid content was estimated by the aluminum chloride colorimetric method described by Muthukumaran et al. [17], using quercetin as the standard in the curve determination.

Acute toxicity test (LD₅₀)

Acute toxicity of aqueous extracts was determined using modified Lorke's [18] approach. Thirteen 20–25-g mice were used in this study. Two stages of mice were used. Nine mice were separated into three groups of three and administered aqueous extracts at 10, 100, and 1000 mg/kg of body weight in phase 1. After 24 hours, mice were monitored for death. The absence of deaths in phase 1 led to greater doses in phase 2. In phase 2, three groups of animals received aqueous extract at 1600, 2900, and 5000 mg/kg. The number of deaths was recorded after 24 hours. The geometric mean of the greatest non-lethal and lowest toxic doses determined the LD₅₀.

Sample size for animal use. Animals were randomly selected, and the sample size was based on the Charan and Kantharia [19] equation: Corrected Sample Size = Sample Size / (1 – [% attrition / 100]). Given a 16.6% attrition and a corrected sample size of 60 mice, the sample size was 60, with each group consisting of 6 animals. To account for potential attrition (death) during the study, one animal was added to each of the groups A–J. Therefore, the total number of mice required in each antimalarial study (suppressive and curative) was 60. Only curative tests are reported in this study.

Experimental Design : The curative properties (Rane's test) of various extract doses were assessed using the procedure described by Misganaw et al. [20]. The mice were injected via the intraperitoneal route with 0.2 ml of a 10-fold dilution (made in normal saline) of blood

Table 1. Summary of extract treatment

Grouping	Sample s/n	Treatment dose (mg/kg)	Number of mice
A (negative) Control	1	10.00 mL/kg of distilled water (DW)	6
B (positive) Control	2	2.30/13.70 mg/kg of Artemether/Lumefantrine (A/L)	6
C	3	190.40 mg/kg of <i>Carica papaya</i> leaf extract	6
D	4	380.79 mg/kg of <i>Carica papaya</i> leaf extract	6
E	5	190.40 mg/kg of <i>Cymbopogon citratus</i> leaf extract	6
F	6	380.79 mg/kg of <i>Cymbopogon citratus</i> leaf extract	6
G	7	190.40 mg/kg of <i>Artemisia annua</i> leaf extract	6
H	8	380.79 mg/kg of <i>Artemisia annua</i> leaf extract	6
I	9	190.40 mg/kg of combined <i>C. papaya</i> , <i>C. citratus</i> and <i>A. annua</i> leaves extract	6
J	10	380.79 mg/kg of combined <i>C. papaya</i> , <i>C. citratus</i> and <i>A. annua</i> leaves extract	6
Total			60

obtained from *P. berghei*-infected mice. On day 3 (D3) following infection, parasitized erythrocytes were evaluated, and the mice were distributed at random into ten (10) groups of six (6) mice each (n=6) is provided in table-1.

Treatment commenced at the end of day 3 and was administered for three consecutive days. At the end of day 7, blood samples were collected for full blood count.

Preparation of extract stock solution and administration of doses

Extract: A high-dose (380.79 mg/kg) stock (38.08 mg/ml) was formed by dissolving 190.4 mg (0.194 g) of extracts in 5 ml of distilled water. For the low dose (190.4 mg/kg), a double-fold dilution of the high-dose stock was prepared to have a lower concentration (19.04 mg/ml). Using the following procedure, different doses of the extract were administered to the animals according to their body weight: Dose = (Weight of mice (g)) / (1000 g) × dose. Standard dose of Artemether/Lumefantrine (positive control) used for the mice was determined similar to Georgewill et al.[21] and Erhirhie et al.[22]

Dosage selection for anti-malaria study: A 1/10th of the LD50 was used to estimate a safe dose for repeated administration. Thus, 1/10 × 3,807.89 mg = 380.79 mg/kg. The same 389.79 mg/kg was used for samples A, B, and C. Since two doses were used, 1/20th of 3,807.89 mg/kg (190.40 mg/kg) and 1/10th of 3,807.89 mg/kg (380.79 mg/kg) were selected for low and high doses.

Inoculation of mice with *Plasmodium berghei* (NK65)

Inoculation followed a protocol comparable to that of Akinyede et al.[23]. The mice were injected intraperitoneally with 0.2 ml of blood from mice infected with *Plasmodium berghei*, which had been diluted 10-fold in normal saline.

Determination of Parasitemia Level

Thick blood smears in 100% methanol were produced from the blood collected in the EDTA tube to measure parasitaemia percentage. It was air-dried at room temperature before being dyed with 2% Giemsa at pH 7.2 for 15 minutes. The slide was then washed with distilled water. Allow to air dry for 15 minutes. They were examined microscopically for parasites after immersion in oil [x100 magnification].



Percentage parasitaemia levels were calculated by counting the number of infected erythrocytes from five fields using the microscope's x100 objective.

Hematological Parameters

An automated haematology analyser was used in measuring a wide range of parameters: for red blood cells (RBC), haemoglobin (Hb), haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW); white blood cells (WBC) and differential such as neutrophils, lymphocytes, monocytes, eosinophils, and basophils; and platelets and mean platelet volume (MPV), and platelet distribution width (PDW). The procedure adopted was similar to Jairajpuri et al.[24], and Pinto et al.[25].

Determination of effects of extracts on antioxidant parameters

The colorimetric procedure was used to determine the antioxidant levels of SOD and MDA. The colorimetric method of Beauchamp and Fridovich [26] was used to measure SOD, while the colorimetric method of Okechukwu et al. [27] was used to measure MDA.

Determination of effects of extracts on Liver function parameters

The effects of the extracts on liver function parameters such Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Gamma-glutamyl transferase (GGT), Bilirubin, Albumin & Total Protein were determined using standard methods.

Statistical analysis

The Statistical Package for Social Science (SPSS) version 27 was employed for data analysis. Results were expressed as mean $\pm$ standard deviation. A one-way analysis of variance (ANOVA) was conducted to compare the mean values between A/L and the samples, followed by post-hoc Dunnett's test. A p-value of less than 0.05 was deemed statistically significant, whilst a p-value greater than 0.05 was regarded as statistically nonsignificant.

3.Results and Discussions

Results

Extraction of *C. papaya*, *C. citratus*, and *A. annua*

Table 2 shows percentage yields of various samples of aqueous leaf extracts. A total of 35 g of *Carica papaya* (representing Sample A) was originally used in the extraction. This quantity yielded 10.16 g (29.03%) following aqueous extraction. Aqueous extract started with 70 g of *Cymbopogon citratus* (Sample B). The recovery was 9.72 g, 13.89%. Extractions began with 14.5 g of powdered *Artemisia annua* (sample C). After extraction, 3.07 g was recovered and 21.17 % aqueous yield. After aqueous extraction, 20 g of leaves (sample D) of *Carica papaya*, *Cymbopogon citratus*, and *Artemisia annua* in the ratios of 20:30:50 gave 3.82 g, totalling 19.10% yield.

Qualitative phytochemical analysis of aqueous extracts of *C. papaya*, *C. citratus*, and *A. annua*.

The phytochemical analyses of *C. papaya*, *C. citratus*, *A. annua*, and their combined extracts are shown in Table 3. Million's and Ninhydrin assays confirmed that all samples contained protein, with *C. papaya* having the highest quantity. Minimal carbohydrates were found in

Table 2: Percentage yield of various samples extracted

S/no	Item	Before extraction(g)	After extraction (g)	Percentage yield %,
1.	Aqueous extract of <i>Carica papaya</i> (Freeze drying) (A)	35	10.16	29.03
2.	Aqueous extract of <i>Cymbopogon citratus</i> (Freeze drying) (B)	70	9.72	13.89
3.	Aqueous extract of <i>Artemisia annua</i> (Freeze drying) (C)	14.5	3.07	21.17
4.	Aqueous extract of the combined plants: 20 %, 30 % and 50 % (Freeze drying) (D)	20	3.82	19.10

Table 3: Qualitative analysis of Phytochemicals in aqueous extracts of *C. papaya*, *C. citratus* and *A. annua*.

S/no	Phytochemical	Test	A (<i>C. papaya</i>)	B (<i>C. citratus</i>)	C (<i>A. annua</i>)	D (Combined extracts)
1	Proteins	Millon's test	++	+	+	+
		Ninhydrin test	++	+	+	+
2	Carbohydrates	Iodine test	+	-	+	-
3	Phenols	Litmus test	+	++	+++	++
		Ferric chloride test	+	++	+++	++
4	Tannins	Geletin test	+	++	++	++
5	Flavonoids	Alkaline reagent test	+	++	+++	++
		Shinoda test	+	++	+++	++
6	Saponins	Frothing test	+	+	+	+
7	Glycoside	Keller-kilani test	++	+	+	+
8	Steroids	L-Burchard's test	+	+	+	+
9	Terpenoids	Salkowski's test	+	-	+	++
10	Alkaloids	Wagner's test	+++	+	+++	++

Key: "-" (Absent), "+" (Present in trace), "++" (Present in moderate level), "+++" (Present in abundant level).

all samples. *A. annua* had the greatest phenols, followed by *C. citratus* and the extract. The lowest phenol content was in *C. papaya*. *C. citratus*, *A. annua*, and the combined extract had rather high tannin levels, but *C. papaya* had less. The alkaline reagent test showed flavonoids in *A. annua*, whereas *C. citratus* had a similar quantity to the combined extract. Few flavonoids were found in *C. papaya*. The Shinoda test showed similar flavonoids in samples. However, saponins were few in all samples. Some *C. papaya* samples had glycosides, but others did not. The samples also exhibited low steroid levels. Terpenoids were significant in the combined extract but low in *C. papaya* and *A. annua* and absent in *C. citratus*. *C. papaya* and *A. annua* had several alkaloids. The

combined extract is modest, while *C. citratus* has the lowest alkaloids.

Acute toxicity (LD₅₀) testing of the combined aqueous extracts of *C. papaya*, *C. citratus* and *A. annua*

Three mice were examined for the acute toxicity (LD₅₀) of sample D, which contained *C. papaya* (20), *C. citratus* (30), and *A. annua* (50). Three mice received 10 mg/kg, 100 mg/kg, and 1000 mg/kg in phase 1. In phase II, mice received 1600, 2900, and 5000 mg/kg of the combined extract. However, mice given 5000 mg/kg became weak after 24 hours and recovered later. The geometric mean of the greatest non-lethal dose (2,900 mg/kg) and the least toxic dose (5,000 mg/kg) was 3,807.89

Table 4. Acute toxicity test results the combined aqueous leaf extracts

Phase	Sample	Dose (mg/kg)	Number of Survived mice	Signs of toxicity
Phase 1	Combined extracts	10	0/3	Nil
		100	0/3	Nil
		1000	0/3	Nil
Phase 2	Combined extracts	1600	0/2	Nil
		2900	0/1	Nil
		5000	0/1	Weakness

Table 5. Curative effects of the aqueous extract of *C. papaya*, *C. citratus*, *A. annua* and combination treatment on parasitemia

Treatment	Grouping	Parasitemia count (day 3)	Parasitemia count (day 7)	Parasitemia curative (%)
Negative control (Distilled water, 10 ml/kg),	A	19.17 ± 2.04	19.17 ± 2.04	0.00
Positive control (Artemether/Lumefantrine, 2.3/13.7mg/kg)	B	0.00 ± 0.00	0.00 ± 0.00*	100.00
Test 1 (190.40 mg/kg of <i>Carica papaya</i>)	C	11.67 ± 2.58	8.33 ± 4.08*	56.53
Test 2 (380.79 mg/kg of <i>Carica papaya</i>)	D	9.17 ± 2.04	7.50 ± 1.22*	60.88
Test 3 (190.40 mg/kg of <i>Cymbopogon citratus</i>)	E	8.00 ± 2.74	10.00 ± 0.00*	47.84
Test 4 (380.79 mg/kg of <i>Cymbopogon citratus</i>),	F	10.00 ± 0.00	7.00 ± 2.74*	63.48
Test 5 (190.40 mg/kg of <i>Artemisia annua</i>),	G	7.00 ± 2.74	7.40 ± 2.51*	61.40
Test 6 (380.79 mg/kg of <i>Artemisia annua</i>),	H	7.50 ± 2.74	8.33 ± 2.58*	56.53
Test 7 (190.40 mg/kg of combined aqueous extracts (CCA),	I	8.75 ± 2.50	8.00 ± 2.45*	58.27
Test 8 (380.79 mg/kg of combined aqueous extracts (CCA).	J	7.50 ± 2.89	10.00 ± 0.00*	47.84

Values are presented as mean ± standard deviation, n = 6. ^{ns}P > 0.05: Not statistically significantly different from control group (distilled water). *P < 0.05: Statistically significantly different from control group (distilled water).),

mg/kg for the combined extract (*C. papaya* 20%, *C. citratus* 30%, *A. annua* 50%). LD₅₀ for sample D was determined to be 3,807.89 mg/kg.

Curative effects of combined aqueous extract on mice parasitemia and hematological parameters.

Table 5 illustrates the curative efficacy of aqueous extracts from *Carica papaya*, *Cymbopogon citratus*, *Artemisia annua*, and their combinations on mice with parasitemia. The positive control using artemisinin combination therapy eradicated parasitemia completely on days 3 and 7, while the negative control (distilled water) showed no significant change. On day 7, significant dose-dependent

therapeutic effects were observed in all test groups compared to the standard treatment. Specifically, groups administered *Cymbopogon citratus*, *Artemisia annua*, and *Carica papaya* exhibited comparable curative rates of approximately 60-64%. The combined extract group showed a curative effect of 58.77%, while groups given only *Carica papaya* and *Artemisia annua* had the lowest curative percentages at 47.84%.

Table 6. Curative effects of aqueous extracts of *C. papaya*, *C. citratus*, *A. annua* and Combined extracts on hematological parameters.

Treatment	RBC (x10 ⁹ /L)	HBG (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelet (x10 ⁹ /L)
Negative control (A)	6.07 ± 0.68	23.32 ± 36.10	31.38 ± 2.38	51.97 ± 4.14	14.50 ± 0.79	27.88 ± 1.35	326.83 ± 210.52
Positive control (B)	6.09 ± 2.30 ^{ns}	8.70 ± 3.29 ^{ns}	32.12 ± 11.70 ^{ns}	53.18 ± 3.64 ^{ns}	14.24 ± 0.54 ^{ns}	26.70 ± 1.59 ^{ns}	508.40 ± 421.20 ^{ns}
Test 1 (C)	5.84 ± 2.07 ^{ns}	7.65 ± 2.43 ^{ns}	29.92 ± 9.65 ^{ns}	56.63 ± 4.23 ^{ns}	14.50 ± 0.75 ^{ns}	25.52 ± 1.51*	194.00 ± 110.19 ^{ns}
Test 2 (D)	7.28 ± 2.07 ^{ns}	10.38 ± 2.83 ^{ns}	41.88 ± 11.31 ^{ns}	57.90 ± 3.86 ^{ns}	14.45 ± 1.09 ^{ns}	24.95 ± 1.11*	383.67 ± 314.66 ^{ns}
Test 3 (E)	5.08 ± 2.12 ^{ns}	7.68 ± 3.21 ^{ns}	29.74 ± 13.16 ^{ns}	57.24 ± 3.61 ^{ns}	14.82 ± 0.47 ^{ns}	26.26 ± 1.43 ^{ns}	405.20 ± 418.63 ^{ns}
Test 4 (F)	6.19 ± 1.06 ^{ns}	8.76 ± 0.88 ^{ns}	32.78 ± 3.95 ^{ns}	54.04 ± 1.88 ^{ns}	14.48 ± 0.87 ^{ns}	26.46 ± 0.92 ^{ns}	308.40 ± 200.13 ^{ns}
Test 5 (G)	6.73 ± 0.83 ^{ns}	9.72 ± 0.72 ^{ns}	35.32 ± 3.77 ^{ns}	48.30 ± 2.86 ^{ns}	13.84 ± 0.72 ^{ns}	27.38 ± 1.93 ^{ns}	584.20 ± 335.11 ^{ns}
Test 6 (H)	5.37 ± 2.73 ^{ns}	7.28 ± 4.00 ^{ns}	27.62 ± 14.43 ^{ns}	57.20 ± 4.00 ^{ns}	14.85 ± 0.89 ^{ns}	25.97 ± 1.62 ^{ns}	163.33 ± 114.72 ^{ns}
Test 7 (I)	7.46 ± 1.75 ^{ns}	10.68 ± 2.61 ^{ns}	42.78 ± 9.48 ^{ns}	56.48 ± 3.60 ^{ns}	14.33 ± 0.35 ^{ns}	24.93 ± 0.74*	371.00 ± 338.68 ^{ns}
Test 8 (J)	4.72 ± 2.25 ^{ns}	7.15 ± 3.45 ^{ns}	28.13 ± 14.19 ^{ns}	58.13 ± 4.45 ^{ns}	15.00 ± 0.67 ^{ns}	25.93 ± 1.82 ^{ns}	428.50 ± 396.76 ^{ns}

Values are presented as mean ± standard deviation, n = 6. ^{ns}P > 0.05: Not statistically significantly different from control group (distilled water). *P < 0.05: Statistically significantly different from control group (distilled water). Negative control (Distilled water, 10 ml/kg), Positive control (Artemether/Lumefantrine, 2.3/13.7mg/kg)

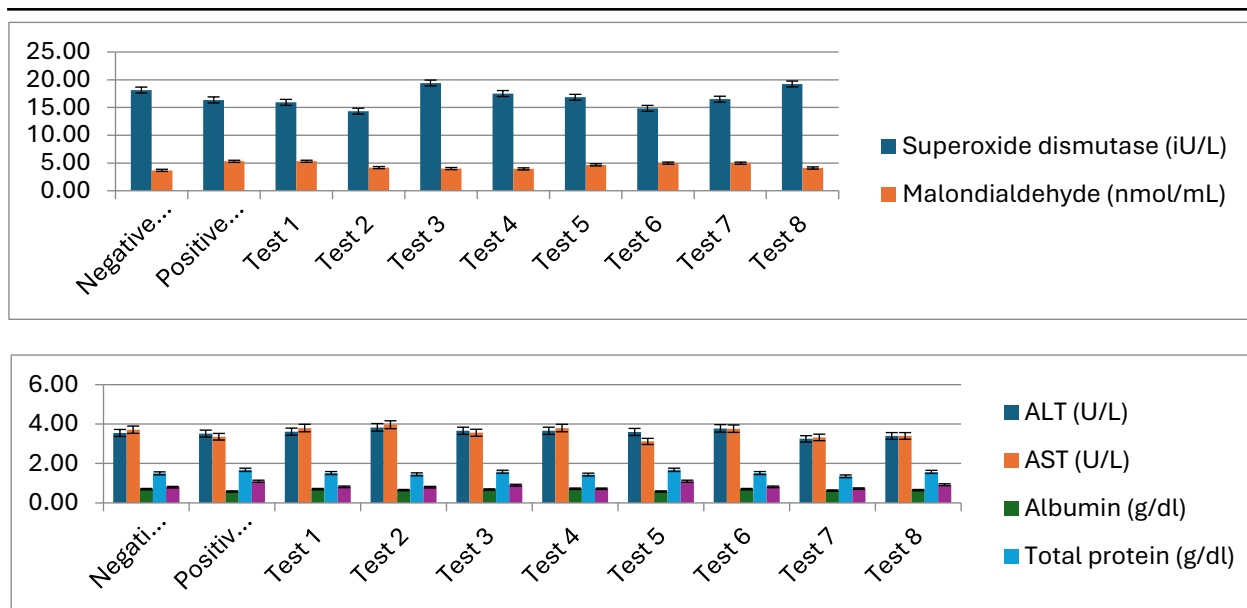
Effects of aqueous extract of *C. papaya*, *C. citratus* and *A. annua* on oxidative stress activities in mice.

Figure 1 depicts the antioxidant effects of aqueous extracts from *C. papaya*, *C. citratus*, and *A. annua* on mice parasitemia after 7 days of therapy. The positive control with artemether and lumefantrine had increased MDA levels (5.30 ± 0.95*), similar to test 1 with 190.40 mg/kg *C. papaya* (5.31 ± 1.05*). Both groups showed substantial differences (p < 0.05) from the negative control (distilled water) and other test groups. In addition, the test 2 group (380.79 mg/kg *C. papaya*) showed a significant decrease in SOD levels (14.34 ± 1.58*)

compared to controls and all other groups (1, 3-8) (p < 0.05).

Figure 1. Effects of extracts on antioxidant parameters

Values are presented as mean ± standard deviation, n = 6. ^{ns}P > 0.05: Not statistically significantly different from control group (distilled water). *P < 0.05: Statistically significantly different from control group (distilled water). Negative control (Distilled water, 10 ml/kg), Positive control (Artemether/Lumefantrine, 2.3/13.7 mg/kg).



Effects of extracts on liver function parameters of mice infected with plasmodium berghei

The study indicates that treatment with *C. papaya*, *C. citratus*, and *A. annua* resulted in no statistically significant differences ($p > 0.05$) in average levels of ALT, AST, albumin, ALP, and total protein between the positive control groups treated with artemether and lumefantrine and the control group. Additionally, no significant differences were observed in total bilirubin, direct bilirubin, globulin, and GGT levels ($nsp > 0.05$) when comparing these groups to the control.

Figure 2. Curative effect of aqueous extract of *C. papaya*, *C. citratus* and *A. annua* on liver biomarkers in mice.

Values are presented as mean $\pm$ standard deviation, $n = 6$. $^{ns}P > 0.05$: Not statistically significantly different from control group (distilled water).

Discussion

Qualitative phytochemical analysis reveals that *C. papaya*, *C. citratus*, and *A. annua* exhibit antimalarial properties aligned with earlier studies. *A. annua* is noted for its artemisinin content and synergistic phytochemicals,

notably flavonoids and phenols, which enhance its effects. *C. papaya*, while less studied, contains alkaloids that may support antimalarial activity and host defense [28]. *C. citratus* contributes with phenols and flavonoids but lacks substantial alkaloids, indicating a supportive role in herbal formulations. Overall, *A. annua*'s phytochemical profile underpins its antimalarial capabilities while *C. papaya* and *C. citratus* provide complementary benefits, suggesting potential for combined extracts to enhance efficacy against malaria.

Acute toxicity testing of aqueous extracts from *C. papaya*, *C. citratus*, and *A. annua* revealed an LD₅₀ of 3,807.89 mg/kg, indicating moderate toxicity. No mortality was observed even at the highest dose of 5,000 mg/kg, with only transient weakness in mice, which was reversible. The study suggests a wide safety margin for acute exposure [29], but advises caution and the need for further toxicity evaluations, including sub-chronic and chronic studies, for a thorough risk assessment. These findings are consistent with research in herbal medicine, highlighting the importance of determining safe dosage limits for clinical use. In figure 5. the study showed that aqueous extracts from *C. papaya*, *C. citratus*, *A. annua*, and their combination extracts reduced



parasitemia in mice. Positive control artemisinin/lumefantrine treatment cleared 100% parasites by days 3 and 7. This results agrees with study on humans using artemether and lumefantrine as reported by Ippolito et al [30]. Parasitemia did not decrease in the negative control group treated with distilled water, demonstrating ongoing infection. The aqueous extracts considerably decreased parasitemia on day 7 compared to the positive control. In particular, test groups receiving 380.79 mg/kg of *C. citratus*, 190.40 mg/kg of *A. annua*, and 380.79 mg/kg of *C. papaya* exhibited parasitemia levels around 7 and curative effects of 61–64%. These plants may be beneficial as individual therapies in ethnomedicine and resource-limited settings because of their antimalarial curative percentages [31]. Clinical validation and regulated dosage are required for widespread use. At 190.40 mg/kg and 380.79 mg/kg dosages, combined extracts demonstrated lower curative rates (47.84 to 58.77%) than solo extracts, demonstrating no synergy.

The lack of synergistic effects in the combined extract at the 20:30:50 ratio (*Carica papaya*: *Cymbopogon citratus*: *Artemisia annua*) is likely due to antagonistic phytochemical interactions, suboptimal dosing, or pharmacokinetic incompatibilities in the *P. berghei* rat model. The justification for using dosing ratios of the extract: *C. papaya* (20), *C. citratus* (30), and *A. annua* (50) is to eliminate resistance and reduce the quantity of artemether, which is more costly to produce. Moreover, synergistic effects were not achieved because of possible phytochemical antagonism. This is because *C. papaya*'s carpine/alkaloids may compete with *Artemisia*'s artemisinin sesquiterpenes for CYP450 metabolism, reducing efficacy; *C. citratus* essential oils potentially inhibit active compound uptake [32]. Bioavailability issues remained an important fact since aqueous extracts lose lipophilic activity, while the liver stage of *P. berghei* is less responsive than the blood

stage of *P. falciparum*, as reported by Melariri et al. [33]. Future formulations with lipid medium or solvent might yield better synergistic activity since artemisinin-herb synergies require lipid formulations [34].

Moreover, Atanu et al. [35] found antimalarial properties in *C. papaya* and other therapeutic plants. Similar to antimalarials, *C. papaya* extracts decrease parasitemia and increase Plasmodium-infected mouse survival. *C. citratus* and *A. annua* are likewise antiplasmodial, supporting their use in traditional antimalarials. Due to dose-dependent effects and parasitemia cure, these herbs may be alternative or additional antimalarials.

4. Conclusion

The acute toxicity assessment suggests moderate safety for the combined extracts, recommending therapeutic usage with judicious dosing. These extracts reduced parasitemia in Plasmodium-infected mice, indicating antimalarial potential. Extracts did not cause hepatotoxicity or oxidative stress, sustaining liver function and antioxidant activity. Haematological tests showed malaria-related anaemia management safety and possible advantages. The results confirm the historic usage of these plants in herbal medicine and suggest they may be effective malaria treatments in resource-limited situations. For therapeutic optimisation, future research should focus on clinical validation and uniform dose.

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Conflict of Interest. There is none among the authors

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Ethics Statement

Ethical approval with numbers **NAU/AREC/2025/0105** was obtained from the Animal Research Ethics Committee (AREC) on the use of animals' policy of Nnamdi Azikiwe University, Awka. Additionally, all other protocols in the study strictly followed the National Council on Research(NRC)[36]guidelines for research involving the use of animals.

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