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ANTIMALARIAL AND ANTIPLASMODIAL ACTIVITIES OF EXTRACTS AND FRACTIONS *OF ANACARDIUM OCCIDENTALE* L. IN MICE EXPERIMENTALLY INFECTED WITH *PLASMODIUM BERGHEI*

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ABSTRACT

Malaria remains a major public health challenge, particularly in sub-Saharan Africa, where increasing resistance of Plasmodium parasites to existing antimalarial drugs necessitates the search for new therapeutic agents. This study evaluated the antimalarial and antiplasmodial activities of the ethanol leaf extract and solvent fractions of *Anacardium occidentale* in Swiss albino mice experimentally infected with *Plasmodium berghei.* Fresh leaves of *A. occidentale* were extracted with ethanol and fractionated into n-hexane, chloroform, ethyl acetate, n-butanol, and aqueous fractions. Qualitative phytochemical screening and acute toxicity studies were performed using standard procedures. The crude extract was administered orally at doses of 100, 200, and 250 mg/kg body weight, while each solvent fraction was administered at 200 mg/kg body weight. Antiplasmodial activity was evaluated using suppressive (4-day test), prophylactic, and curative models. Chloroquine (5 mg/kg) and pyrimethamine (1.2 mg/kg)

served as standard drugs. Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with significance set at $p < 0.05$. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, combined anthraquinones, and phlobatannins. No mortality or signs of toxicity were observed in mice administered up to 5000 mg/kg of the crude extract. The crude extract exhibited dose-dependent antiplasmodial activity, with chemosuppression increasing from 29.90% at 100 mg/kg to 58.86% at 250 mg/kg. Among the solvent fractions, the ethyl acetate fraction demonstrated the highest suppressive (72.32%) and prophylactic (65.66%) activities, whereas the n-hexane fraction produced the greatest curative effect and longest mean survival time (17.10 ± 0.26 days). Chloroquine and pyrimethamine exhibited significantly higher antiplasmodial activities than all extract-treated groups ($p < 0.05$). The findings demonstrate that the ethanol leaf extract and solvent fractions of *A. occidentale* possess significant antimalarial and antiplasmodial activities against *P. berghei* infection. The ethyl acetate fraction showed the most potent suppressive and prophylactic effects, while the n-hexane fraction exhibited superior curative activity. These results support the traditional use of *A. occidentale* in malaria treatment and suggest that the plant may serve as a promising source of bioactive compounds for antimalarial drug development.

KEYWORDS: *Anacardium occidentale*, antimalarial activity, antiplasmodial activity, Extracts, Fraction, *Plasmodium berghei*.

INTRODUCTION

Malaria remains one of the most important parasitic diseases affecting humans worldwide. It is caused by protozoan parasites of the genus *Plasmodium*, with five species known to infect humans. Among these, *Plasmodium falciparum* is responsible for the majority of severe and fatal malaria cases, whereas *Plasmodium vivax* is associated with recurrent infections due to the persistence of dormant liver-stage parasites.[1,2]

Despite decades of intensive control efforts, malaria continues to pose a substantial public health challenge, particularly in tropical and subtropical regions. According to the World Health Organization (WHO), there were an estimated 282 million malaria cases and approximately 610,000 malaria-related deaths globally in 2024. The WHO African Region remains disproportionately affected, accounting for about 95% of global malaria cases and deaths, with children under five years of age representing approximately 75% of malaria-related mortality in the region. Nigeria continues to bear the greatest malaria burden

worldwide, contributing approximately 32% of malaria deaths in the African region and remaining one of the principal countries driving global malaria morbidity and mortality.[2]

Although significant progress has been achieved through the deployment of insecticide-treated bed nets, indoor residual spraying, improved diagnostics, artemisinin-based combination therapies (ACTs), and recently introduced malaria vaccines, the disease remains difficult to control. Factors such as drug-resistant parasites, insecticide-resistant mosquito vectors, climate change, population displacement, and inadequate healthcare access continue to undermine malaria control programmes worldwide.[2,3]

The emergence and spread of resistance to conventional antimalarial drugs, particularly among *P. falciparum* strains, constitute one of the greatest threats to malaria elimination efforts. Resistance to previously effective drugs has reduced treatment efficacy and highlighted the urgent need for the discovery of novel antimalarial agents with new mechanisms of action.[4,5] Consequently, the search for alternative therapeutic compounds from natural sources has intensified in recent years.

Medicinal plants have historically played a pivotal role in antimalarial drug discovery. Classical examples include quinine, isolated from *Cinchona* species, and artemisinin, obtained from *Artemisia annua*, both of which have contributed significantly to malaria treatment and control.[6] Furthermore, numerous plant species employed in traditional medicine have demonstrated promising antiplasmodial activities, indicating that natural products remain a valuable source of novel lead compounds for antimalarial drug development.[7]

Anacardium occidentale L. (Anacardiaceae), commonly known as the cashew tree, is a tropical plant native to Central and South America but now widely cultivated throughout Africa, Asia, and other tropical regions. Beyond its economic significance as a food crop, *A. occidentale* has been extensively utilized in traditional medicine for the management of several ailments, including diabetes mellitus, ulcers, infections, inflammatory disorders, and cancer.[19,20,21] Phytochemical investigations have revealed that the plant contains diverse bioactive constituents such as flavonoids, tannins, alkaloids, terpenoids, phenolic compounds, saponins, and anacardic acid derivatives, many of which possess antimicrobial, antioxidant, anti-inflammatory, and antiparasitic properties.[28]

Recent studies have reported significant antiplasmodial activities of *A. occidentale* extracts against both rodent and human malaria parasites. Methanol and hydro-alcoholic leaf extracts have demonstrated substantial suppressive activity against *Plasmodium berghei* and inhibitory effects against *P. falciparum* metabolic pathways, suggesting that the plant may represent a promising source of antimalarial compounds.[22,23] However, information regarding the comparative antimalarial efficacy of different solvent fractions of *A. occidentale* remains limited.

Therefore, the present study was designed to evaluate the antimalarial and antiplasmodial activities of the crude ethanol extract and solvent fractions of *Anacardium occidentale* leaves in Swiss albino mice experimentally infected with *Plasmodium berghei*. The study further sought to compare the suppressive, prophylactic, and curative activities of the extract and its fractions, with the aim of identifying the most active fraction and providing scientific evidence to support the traditional use of the plant in malaria management.

METHODOLOGY

Collection and Identification of Plant Materials

Fresh leaves of *Anacardium occidentale* were collected from Nduetong Oku, Uyo Local Government Area, Akwa Ibom State, Nigeria. Plant samples were transported in polythene bags to the Department of Pharmacy, University of Uyo, Nigeria for identification. The plant specimen was identified by a taxonomist, voucher number: **UUPH NO. 3(a)** was assigned to the plant and voucher specimen was kept in the Herbarium unit, Department of Pharmacy, University of Uyo, Nigeria.

Preparation of Crude Extracts

The experiment was carried out in the Department of Pharmacology and Toxicology Laboratory, University of Uyo, Nigeria. The crude extract of *Anacardium occidentale* was prepared using cold maceration method with ethanol solvent as described by Harborne (1998) and Sofowora (1993). The concentrated crude extracts were air-dried to constant weight and was used for solvent fractionation.[15,18]

Fractionation of Crude Extracts

Solvent partitioning was carried out to obtain fractions of increasing polarity as described by Harborne (1998) and Sofowora (2008). A measured quantity of each crude extract was dissolved in distilled water and was successively partitioned using separating funnels with n-

hexane, ethyl acetate, chloroform and n-butanol. Each solvent fraction was collected separately, while the remaining aqueous fraction was retained. The collected fractions were concentrated under reduced pressure using a rotary evaporator and were dried to obtain solid residues.[15,18]

Phytochemical Screening

Qualitative phytochemical screening was performed on each solvent fraction to determine the distribution of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds. Standard phytochemical methods described by Harborne (1998) and Trease and Evans (2009) were adopted.[15,17]

Experimental Animals

Two hundred mice of both sexes weighing between 20 - 30g were obtained from The Laboratory Animal Unit of Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The animals were maintained in accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* (DHHS, NIH Publication No. 85-23, 1985). They were kept for a period of 7 days to acclimatize the environment before the commencement of the experiments. They were allowed to have access to clean drinking water and feed (Vital Feed) *ad libitum* before and during the experiments.[16]

Parasite/Test Organism

A chloroquine-sensitive strain of *Plasmodium berghei* obtained from National Institute of Medical Research **(NIMR),** Yaba, Lagos was maintained in Swiss albino mice through serial passage. Donor mice with established parasitemia were used as the source of infected blood for the assay.

Determination of Acute Toxicity of crude *Anacardium occidentale* Extract

Acute toxicity of the crude extract in Swiss albino mice model as determined according to the method described by Lorke (1983). Mice were administered with different doses of the extract of *A. occidentale* (1000 - 5000 mg/kg) intraperitonially to groups of mice (3 mice per group). The animals were monitored daily for manifestation of physical signs of toxicity including writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of mortalities in each group was recorded at 24 hrs intervals.[8]

Parasite inoculation

Parasite inoculation was done based on the method described by Odetola and Basir (1980) as reported by Okokon *et al*. (2017). Briefly, each mouse used in the experiment was inoculated intraperitoneally with 0.2mL of infected blood containing about $1x10^7$ *P. berghei berghei* parasitized erythrocytes. The inoculum consisted of $5x10^7$ *P. berghei berghei* erythrocytes per mL. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations.[9,12]

Drug administration

The drug (Chloroquine and Pyrimethamine) and extract used in the in vivo antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

Evaluation of in vivo anti-malarial activity of ethanol crude Anacardium occidentale extract

Evaluation of suppressive activity of the extract (4-day test)

This test was used to evaluate the schizontocidal activity of the extracts, fractions and Chloroquine against early *P. berghei berghei* infection in mice. This was done according to the method described by Knight and Peters (1980).[13] Fifty (50) mice were randomly divided into ten groups of five mice each. On the first day (D1), All the mice in groups were infected with the parasite and randomly divided into various groups. They were orally administered with the extract, fractions and Chloroquine. The mice in group 1 were administered 100 mg/kg, group 2, 200 mg/kg and group 3, 250 mg/kg of crude *A. occidentalle* extract, while group 4 were administered with fractions of n-Hexane 200mg/kg, group 5, administered fractions of chloroform 200mg/kg, group 6, ethyl-acetate 200mg/kg, group 7, fraction of n-butanol 200mg/kg, group 8, aqueous fractions 200mg/kg, 9 was administered 5 mg/kg of Chloroquine (positive control), and 10 mL/kg of distilled water was administered to group 10 (negative control) for four consecutive days (D0–D3) between 8 am and 9 am. On the fifth day (D4), thin blood film was made from tail blood of each mouse. The film was then stained with Giemsa stain to reveal parasitized erythocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls as follows.

Average % parasitaemia in negative control

$$- \frac{\text{Average \% parasitaemiain Positive groups}}{\text{Average \% parasitaemia in negative control}} \times 100$$

Evaluation of prophylactic or repository activities of *Anacardium occidentale* extract

The repository activities of the extract, fractions, and pyrimethamine were assessed by using the method described by Peters (1965).[10] Fifty (50) mice were randomly divided into ten groups of five mice each. On the first day (D0), All the mice in groups were randomly divided into various groups. They were orally administered with the extract, fractions and Pyrimethamine. The mice in group 1 were administered with 100 mg/kg, group 2, 200 mg/kg and group 3, 250 mg/kg of crude *A. occidentalle* extract, while group 4 were administered with fractions of n-Hexane 200mg/kg, group 5, administered fractions of chloroform 200mg/kg, group 6, fractions of ethyl-acetate 200mg/kg, group 7, fraction of n-butanol 200mg/kg, group 8, aqueous fractions 200mg/kg, 9 was administered 1.2 mg/kg of Pyrimethamine (positive control), and 10 mL/kg of distilled water was administered to group 10 (negative control). Administration of the extract/drug continued for three consecutive days (D0–D2). On the fourth day (D3), the mice were inoculated with *P. berghei berghei*. The parasitaemia level of each mouse was assessed by blood smear after 72 hrs.

Evaluation of curative activities of extract (Rane's test)

This was used to evaluate the schizontocidal activity of the extract, fractions, and Chloroquine in established infection. This was done as described by Ryley and Peters (1970).[11] *Plasmodium berghei berghei* was injected intraperitoneally into another 50 mice on the first day (D0). Seventy-two hours later (D3), the mice were divided randomly into Ten groups of five mice each.

Mice were administered with different doses of extracts 100, 200, and 250 mg/kg to group 1-3, while group 4 -8 were administered with 200mg/kg fractions of n-Hexane, chloroform, ethyl-acetate, n-butanol, and aqueous fractions respectively, group 9 was administered 5 mg/kg of Chloroquine (positive control), and 10 mL/kg of distilled water was administered to group 10 (negative control).

The extract and drugs were administered once daily for 5 days. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 33 days (D0–D33).

$$\frac{\text{No of days survived}}{\text{Total No: of days}} \times 100 = \text{MST}$$

Statistical Analysis

Data obtained from this study were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Differences between treatment groups were considered statistically significant at $p < 0.05$ and 0.001. Statistical analyses were conducted using SPSS, version 2021.

RESULT

Determination of acute toxicity of *Anacardium occidentale* crude extract

Mice in groups did not record any mortality at 5000mg/kg. No physical signs of toxicity such excitation, paw licking, increased respiratory rate, decreased motor activity, gasping or coma was observed when the highest tested dose of 5000mg/kg was administered.

Phytochemical Screening Result of *Anacardium occidentale*

Phytochemical screening results of ***A. occidentale*** is presented in Table 1. The Screening of the leaf of *A. occidentale* revealed the presence of various phytochemical components. Phytochemical screening the presence of Alkaloids, Flavonoids, Cardiac glycosides, Saponins, Tannins and Terpenes, Combined Anthraquinones, and Phlobatannins but free Anthraquinones were absent (Table 1)**.**

Table 1: Phytochemical Constituents of the Leaf of *Anacardium occidentale.*

Plant Metabolite/ Phyto Constituent	Test	
Alkaloids	Dragedroffs	+
Anthraquinones	Borntrager's	-
Combined Anthraquinones	Borntrager's	+
Cardiac glycosides	Salkowski's	+
Flavonoids	Shinoda's reduction test	+
Saponins	Froth	++
	Sodium bicarbonate	+
Tannins	Ferric chloride	++
Phlobatannins	Hydrochloric acid test	+
Terpenes	Liebermann Burchard	+
Carbohydrates	Moliscoh's test	+

+ =Detected, - = Not detected

Effect of suppressive activity of crude ethanol extract and fractions of *Anacardium occidentale* (4-day test) on *P. berghei* infected mice

The suppressive test recorded a dose-dependent antiplasmodial activity of the crude extract of *Anacardium occidentale*, with statistically significant differences observed across treatment groups ($p < 0.05$). The crude extract at 100 mg/kg showed relatively low chemosuppression (29.9%) and a shorter MST (9.80 ± 0.76 days), which was significantly different from higher doses and most solvent fractions. At 200 mg/kg, there was a moderate but significant increase in activity (45.58% suppression; MST = 10.99 ± 1.02 days), while the 250 mg/kg dose exhibited a significantly higher chemosuppressive effect (58.86%) and prolonged MST (15.48 ± 0.96 days), indicating improved efficacy with increasing dose.

Among the fractions, the ethyl-acetate fraction exhibited the highest chemosuppression (72.32%) and significantly prolonged MST (21.22 ± 1.20 days), which was statistically comparable to the standard drug but significantly higher than all other extract fractions ($p < 0.05$). The chloroform fraction also showed strong activity (60.46% suppression), which was significantly higher than the crude extract at lower doses but lower than the ethyl-acetate fraction. The n-hexane fraction demonstrated moderate suppression (45.67%) but produced a relatively high MST (18.46 ± 1.20 days), suggesting a statistically significant improvement in survival compared to several other fractions despite moderate parasite clearance.

In contrast, the n-butanol and aqueous fractions showed significantly lower chemosuppression and MST values compared to the more active fractions, indicating weak antiplasmodial activity. The standard drug, chloroquine, exhibited the highest chemosuppression (82.56%) and longest MST (31.28 ± 0.52 days), which was significantly superior to all extract-treated groups ($p < 0.05$). The untreated control group recorded the highest parasitaemia and shortest MST, significantly differing from all treatment groups.

Table 2: Suppressive anti-plasmodial effect of crude extract of *Anacardium occidentale* and fractions in mice experimentally infected with *Plasmodium berghei*.

TREATMENT	CONC. (mg/Kg)	PARASITAEMIA	Chemosuppression (%)	MST
Crude Extract	100	20.19±0.99	29.9	9.80±0.76
	200	14.57±1.72b	45.58	10.99±1.02
	250	12.56±1.87c	58.86	15.48±0.96b
n-hexane	200	16.22±0.28a	45.67	18.46±1.20a
Chloroform	200	13.04±0.83c	60.46	13.00±0.44a
Ethyl-acetate	200	8.73±1.20a	72.32	21.22±1.20b
n-butanol	200	17.93±0.84a	39.76	12.44±1.32a

Aqueous	200	20.96±2.48	26.8	11.02±0.28a
Chloroquine	5	5.02±0.66c	82.56	31.28±0.52c
Control	-	33.73±1.64	-	8.12±1.33

Values are expressed as mean ±SEM. Significant relative to control [a]p< 0.05; [b]p< 0.01; [c]p< 0.001

Repository/Prophylactic activity of ethanol extract and Fractions of *Anacardium occidentale* against *Plasmodium berghei* infection in mice

In the prophylactic model, statistically significant differences ($p < 0.05$) were observed in the ability of treatments to prevent infection establishment. The crude extract showed a significant dose-dependent increase in chemosuppression, with the 250 mg/kg dose (59.50%) producing a significantly higher effect compared to 100 mg/kg and 200 mg/kg doses. This was accompanied by a significantly longer MST (20.66 ± 3.51 days).

The ethyl-acetate fraction again demonstrated the highest prophylactic activity among the extracts, with 65.66% chemosuppression and an MST of 20.28 ± 0.96 days, which was significantly higher than most other fractions ($p < 0.05$), though still lower than the standard drug. The n-hexane fraction showed comparable activity (58.53% suppression; MST = 20.33 ± 1.55 days), with no statistically significant difference between it and the high-dose crude extract in some comparisons.

The chloroform fraction showed moderate but significantly lower activity compared to ethyl-acetate, while the n-butanol and aqueous fractions exhibited significantly poor prophylactic effects, with low chemosuppression and shorter survival times.

Pyrimethamine produced the highest chemosuppression (81.40%) and MST (26.11 ± 1.22 days), which were significantly greater than all extract-treated groups ($p < 0.05$). The control group recorded significantly higher parasitaemia and lower MST, confirming the absence of prophylactic protection.

Table 3: Repository/Prophylactic activity of ethanol extract and Fractions of *Anacardium occidentale* on *Plasmodium berghei* experimentally infected in mice.

Treatment	Conc. (mg/kg)	Parasitaemia (mean ±SEM)	Chemosuppression (%)	MST
Crude Extracts	100	21.42±1.65	24.28	13.64±0.44
	200	19.05±0.85a	34.36	15.88±1.56
	250	9.46±1.66c	59.50	20.66±3.51b
n-hexane	200	10.10±1.94c	58.53	20.33±1.55b

Chloroform	200	10.98±0.88c	53.48	15.62±0.58
Ethyl-acetate	200	7.88±0.48c	65.66	20.28±0.96b
n-butanol	200	19.63±0.64	19.72	14.68±1.62
Aqueous	200	21.19±0.32	13.65	12.66±1.72
Pyrimethamine	1.2	4.82±0.20c	81.40	26.11±1.22
Control	-	26.02±0.30	-	8.11±0.44c

Values are expressed as mean ±SEM. Significant relative to control $^{a}p< 0.05$; $^{b}p< 0.01$; $^{c}p< 0.001$.

Curative anti-plasmodial effect of crude and fractions of *Anacardium occidentale* in mice experimentally infected with *Plasmodium berghei*

In the curative model, significant differences ($p < 0.05$) were observed among treatments in their ability to reduce established parasitaemia and prolong survival. The crude extract at 250 mg/kg demonstrated a statistically significant reduction in parasitaemia over time compared to lower doses, along with a longer MST (13.20 ± 0.42 days). However, this effect remained significantly lower than that of the most active fractions and the standard drug.

The n-hexane fraction showed the most consistent curative activity, with a progressive decline in parasitaemia from Day 3 to Day 7 and a significantly higher MST (17.10 ± 0.26 days) compared to most other fractions ($p < 0.05$). This suggests a sustained therapeutic effect. The chloroform fraction also demonstrated a relatively high MST (16.20 ± 0.20 days), although its inability to consistently reduce parasitaemia indicates that its effect may be more supportive than curative, and this difference was statistically evident.

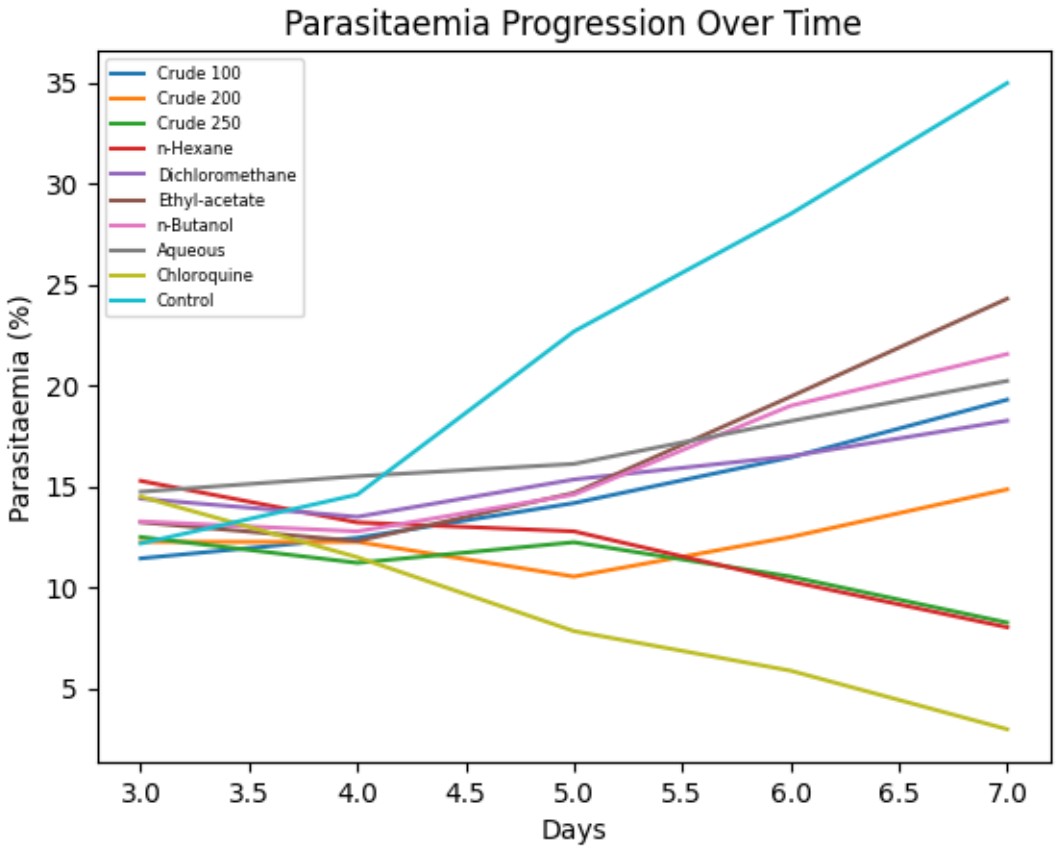
Interestingly, the ethyl-acetate fraction, despite its high suppressive activity, showed a significant increase in parasitaemia toward Day 7 and a comparatively lower MST (12.56 ± 0.50 days), indicating reduced effectiveness in established infections. The n-butanol and aqueous fractions showed significantly poor curative activity, with increasing parasitaemia and lower survival times.

Chloroquine treatment resulted in a statistically significant and progressive reduction in parasitaemia, with the highest MST (30.1 ± 1.3 days), outperforming all extract-treated groups ($p < 0.05$). The control group exhibited a significant increase in parasitaemia and the lowest survival time (6.8 ± 0.12 days).

Table 4: Curative anti-plasmodial effect of crude and fractions of *Anacardium occidentale* in mice experimentally infected with *Plasmodium berghei* (Daily Parasitaemia) (Mean±SEM).

Treatment	DOSE	DAY 3	DAY 4	DAY 5	DAY 6	DAY7	MST
Crude Extracts	100	11.42±0.29	12.44±0.96	14.16±16	16.42±1.92	19.28±0.42c	7.94±6.46
	200	12.24±0.44	12.24±0.44	10.52±0.68	12.48±0.48	14.84±1.30c	11.44±0.78
	250	12.48±0.21	11.20±0.92	12.22±1.28c	10.52±0.38	8.24±1.48c	13.20±0.42a
n-Hexane	200	15.26±0.24	13.20±1.02	12.76±1.68	10.28±1.58	8.01±0.002c	17.10±0.26c
Chloroform	200	14.38±1.88	13.48±0.93	15.33±0.98	16.48±0.96	18.24±0.76c	16.20±0.20b
Ethyl-acetate	200	13.21±56	12.28±0.44	14.66±0.20	19.43±0.92	24.28±1.34	12.56±0.50a
n-Butanol	200	13.25±1.80	12.76±0.63	14.58±0.32	18.98±1.20	21.54±1.48c	8.46±0.58
Aqueous	200	14.72±62	15.49±0.11	16.10±1.02	18.22±48	20.21±1.28c	9.72±0.52
Chloroquine	5	14.48±0.88	11.48±0.44	7.82±0.12c	5.86±0.44c	2.96±0.67c	30.1±1.3
Control	-	12.16±1.02	14.58±0.78	22.66±1.36	28.48±1.26	34.96±1.36c	6.8±0.12c

Values are expressed as mean ±SEM. Significant relative to control [a]p< 0.05; [b]p< 0.01; [c]p< 0.001



DISCUSSION

The result of the acute toxicity study of the extract of *A. occidentale* show no deaths, dypsnea and lethargy. No physical signs of toxicity such excitation, paw licking, increased respiratory

rate, decreased motor activity, gasping or dizziness was observed at 5000 mg/kg. This probably could be that tested plant extracts may contain no toxic compound that could be dangerous to the health of mice at a minimal dosage, or however, it could also suggest that the plant has a wide safety margin in mice. Thus, this report is consistent with the study of Tedong *et al.* (2007) who documented that the plant showed no toxic signs and mortality in mice when administered with 14g/kg, however, a lethal dose of 16g/kg of *A. occidentale* was reported.[14]

The phytochemical investigation carried out in this study involving assays for Akaloids, anthraquinones (free and combined), Cardiac glycosides, Flavonoids, Saponins, Tannins, Phlobatannins and Terpenes, the results obtained corroborated with those in literature. Results obtained from the screening of the leaf extracts of *A. occidentale* agrees with the reports of Tedong *et al.* (2007).[14]

The research systematically evaluated the anti-plasmodial potential of the crude extract and solvent fractions of *Anacardium occidentale* across suppressive, curative, and prophylactic models in *Plasmodium berghei* infected mice, revealing distinct activity profiles that merit comparison with existing literature. The findings confirm the traditional use of this plant for malaria treatment while providing novel insights into the differential efficacy of its solvent fractions across therapeutic models.

The suppressive test results demonstrated a clear dose-dependent antiplasmodial activity of the crude *A. occidentale* extract, with chemosuppression increasing from 29.9% at 100 mg/kg to 58.86% at 250 mg/kg. This dose-response pattern aligns closely with the findings of Tejumade *et al*. (2024), who reported that methanol extracts of *A. occidentale* leaves produced 73.88% chemosuppression at 100 mg/kg in a 4-day suppressive test using the NK65 strain of *P. berghei.*[23] The higher suppression observed in that study at the same dose may be attributable to differences in extraction solvent (methanol versus the present study's crude extract preparation) and strain variability. Notably, the present study's ethyl-acetate fraction exhibited the highest suppressive activity (72.32%), which was statistically significant and comparable to chloroquine (82.56%) and substantially higher than the crude extract at all doses. This observation is consistent with the work of Olanlokun *et al*. (2012), who demonstrated that ethyl acetate fractions of *Alstonia boonei* stem bark exhibited enhanced therapeutic effects against *P. berghei*-induced malaria compared to crude extracts.[25]

The result of suppression of *A. occidentale* also reveals a suppressive superiority of the ethyl-acetate fraction over other solvent fractions (chloroform, n-hexane, n-butanol, aqueous) mirrors findings from fractionation studies on other antimalarial plants. However, Dawet *et al*. (2023) reported that ethyl acetate fractions of *Pseudocedrela kotschyi* stem bark contained 44 bioactive compounds and exhibited significant antimalarial activity in both suppressive and curative tests.[26] Similarly, the ethyl acetate fraction of *Spilanthes filicaulis* demonstrated enhanced antimalarial efficacy against *P. berghei*-infected mice, reinforcing the notion that medium-polarity solvents effectively concentrate antimalarial principles. The moderate activity of the n-hexane fraction (45.67% suppression) coupled with a relatively prolonged mean survival time (MST) of 18.46 days suggests that non-polar constituents may contribute to host-protective effects distinct from direct parasite clearance, a phenomenon also observed with hexane fractions of *Eremostachys macrophylla*, which showed considerable antimalarial activity in vitro.[26]

In the curative model, the n-hexane fraction unexpectedly emerged as the most effective, demonstrating a progressive reduction in parasitaemia from Day 3 to Day 7 and a significantly higher MST (17.10 days) compared to other fractions. This finding contrasts with the suppressive test results, where the ethyl-acetate fraction was superior, and suggests that different phytochemical classes may be preferentially active against established versus nascent infections. The curative efficacy of *A. occidentale* is supported by Sha'a (2014), who reported that aqueous and ethanolic extracts of *A. occidentale* produced significant reductions in parasitaemia and exerted curative effects in *P. berghei*-infected mice, indicating promising antimalarial activities worthy of further exploration.[24] The ethyl-acetate fraction, despite its exceptional suppressive activity, paradoxically showed reduced effectiveness in the curative model, with parasitaemia increasing toward Day 7 and a comparatively lower MST. This pattern suggests that the ethyl-acetate fraction may contain compounds that act primarily on early-stage parasites (schizontocidal activity) but lack sustained efficacy against established infections, a phenomenon also observed with certain fractions of *Prosopis africana*, where methanol extracts showed superior suppression but varying curative profiles depending on the fraction tested.

The chloroform fraction's ability to prolong survival without consistent parasite clearance aligns with the properties of supportive antimalarial agents that modulate host immune responses rather than exerting direct antiparasitic effects. This is reminiscent of the activity of

certain terpenoids and flavonoids that enhance host resistance to infection, as reported in the comprehensive review of Anacardium plants by Salehi *et al*. (2020), which highlighted the immunomodulatory and antioxidant properties of cashew-derived compounds.[28]

The prophylactic model of *A. occidentale* revealed that both the crude extract (250 mg/kg) and ethyl-acetate fraction conferred substantial protection against infection establishment, with chemosuppression values of 59.50% and 65.66%, respectively. These values, while lower than pyrimethamine (81.40 %), are nevertheless clinically meaningful and support the traditional use of *A. occidentale* for malaria prevention. The prophylactic activity of *A. occidentale* is further substantiated by Kaushik *et al*. (2023),[22] who demonstrated that hydro-alcoholic extracts of *A. occidentale* leaves exhibited potent inhibitory activity against recombinant *P. falciparum* transketolase (PfTK), with 75.45% enzyme inhibition and 99.31% growth inhibition of intra-erythrocytic parasites at 50 µg/mL. This enzyme is essential for parasite metabolism, and its inhibition likely contributes to both prophylactic and suppressive effects.

The comparable prophylactic efficacy of the n-hexane fraction (58.53% suppression) to the high-dose crude extract suggests that non-polar constituents also play a role in preventing infection establishment, possibly through mechanisms distinct from those of medium-polarity compounds. The poor prophylactic activity of the n-butanol and aqueous fractions underscores the importance of solvent selection in bioactivity-guided fractionation and is consistent with findings from *Prosopis africana*, where methanol and ethyl acetate extracts showed substantially higher suppression (90% and 73.6%) than more polar fractions.

However, the differential activity profiles observed across solvent fractions of *A. occidentale* can be rationalized by the phytochemical composition of *A. occidentale.* Phytochemical analyses have consistently identified flavonoids, tannins, phenols, alkaloids, and saponins in *A. occidentale* leaves. The ethyl-acetate fraction, which exhibited the highest suppressive and prophylactic activity, is known to concentrate flavonoids and phenolic compounds classes of secondary metabolites with well-documented antimalarial properties. Indeed, fractionation studies of *A. occidentale* leaves from Vietnam revealed that the ethyl acetate fraction contained the highest total phenolic and flavonoid contents and exhibited the most potent bioactivities, including enzyme inhibition and cytotoxicity. The anti-plasmodial activity of *A. occidentale* should be further linked to specific alkyl-phenols, this is supported by studies by Gimenez *et al*. (2019)[29] who reported that ethanol crude extract displayed an IC50 of 0.577

µg/mL against *P. falciparum* and that isolated cardol and cardanol derivatives exhibited IC50 values ranging from 5.39 µM to >100 µM.[29]

The inhibition of β-hematin formation represents another plausible mechanism. Tejumade *et al*. (2024)[23] reported that *A. occidentale* methanol extracts inhibited β-hematin formation with an IC50 of 36.1 µg/mL, a mechanism also exploited by chloroquine and other quinoline antimalarials. The moderate activity of the n-hexane fraction may be attributable to terpenoids and hydrocarbons, which are preferentially extracted by non-polar solvents and have been detected in GC-MS analyses of *A. occidentale* fractions.

The antiplasmodial activity of *A. occidentale* observed in this study compares favorably with other well-investigated antimalarial plants. The ethyl-acetate fraction's suppressive activity (72.32%) is comparable to that reported by Ofeniforo *et al*. (2024)[27] in their study on for ethyl acetate fractions of *Spilanthes filicaulis* (73%) and *Cleome gynandra*, which demonstrated potent antimalarial efficacy and favorable safety profiles.[27] The crude extract's dose-dependent activity aligns with findings for *Psidium guajava*, another plant frequently used in Nigerian traditional medicine for malaria, which showed 72.75% chemosuppression at 100 mg/kg. However, the present study extends these findings by demonstrating that solvent fractionation can yield preparations with efficacy approaching that of standard antimalarial drugs.

However, the findings carry several implications for antimalarial drug discovery. First, the ethyl-acetate fraction emerges as a promising candidate for further bioassay-guided fractionation to isolate and characterize the specific compounds responsible for its potent suppressive and prophylactic activities. Second, the n-hexane fraction's preferential curative activity warrants investigation as a potential source of compounds effective against established, drug-resistant infections. Third, the safety profile of A. occidentale extracts, which have been shown to exhibit no acute toxicity up to 300 mg/kg in mice, supports their continued evaluation as potential therapeutic agents.

Several limitations should be acknowledged. The study employed *P. berghei* ANKA, a rodent malaria parasite that, while widely accepted as a model for human malaria, may not fully recapitulate the pathophysiology of P. falciparum infection. Future studies should validate these findings using human malaria parasites in vitro and, ultimately, in clinical settings. Additionally, the precise mechanisms underlying the differential activity of solvent

fractions across therapeutic models remain to be elucidated through detailed phytochemical characterization and mode-of-action studies.

CONCLUSION

The present study demonstrated that the ethanol leaf extract and solvent fractions of *Anacardium occidentale* possess significant antimalarial and antiplasmodial activities against *Plasmodium berghei* infection in Swiss albino mice. The crude extract exhibited dose-dependent activity in suppressive, prophylactic, and curative models, while the solvent fractions showed varying degrees of efficacy. Among the fractions evaluated, the ethyl acetate fraction produced the highest suppressive and prophylactic activities, whereas the n-hexane fraction demonstrated the most pronounced curative effect and prolonged mean survival time. The extract was found to be relatively safe, with no observable signs of acute toxicity or mortality at doses up to 5000 mg/kg body weight.

The observed antiplasmodial activities may be attributed to the presence of bioactive phytochemicals such as alkaloids, flavonoids, tannins, saponins, terpenoids, and cardiac glycosides detected in the plant. These findings provide scientific support for the traditional use of *A. occidentale* in the management of malaria and suggest that its active fractions may serve as promising sources of novel antimalarial compounds. Further studies involving bioassay-guided isolation, characterization of active constituents, mechanistic investigations, and clinical evaluation are recommended to establish their therapeutic potential.

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