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ORIGINAL ARTICLE

Acute Oral Toxicity, Histopathological Analysis, and Antimalarial Potential of *Jatropha tanjorensis* Leaf Extracts

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Abstract

Background: *Jatropha tanjorensis* is a leafy vegetable widely consumed across Africa and is valued in folk medicine for its reputed blood-replenishing properties. This study aimed to bridge this research gap by assessing the acute oral toxicity, histopathological effects on vital organs, and chemo-suppressive antimalarial activity of *J. tanjorensis* leaf extracts, offering crucial insights into their therapeutic potential and safety profiles.
Methods: Different extracts of *J. tanjorensis* leaves were obtained by sequential extraction via maceration. Acute oral toxicity was assessed using the limit test at a dose of 5000 mg/kg body weight of the experimental mice, following the Organization for Economic Co-operation and Development (OECD) guidelines (425) for rats and mice. Histopathological analysis of the livers and kidneys of mice exposed to the extracts was performed using standard protocols. The chemo-suppressive antimalarial activity was determined using a suppressive test model. Data analysis was conducted using GraphPad Prism version 9.3.1.
Results: The experimental mice exposed to *J. tanjorensis* leaf extracts exhibited no symptoms of toxicity, although one death was recorded across two groups. According to OECD guidelines, with fewer than three deaths observed, the LD_{50} of *J. tanjorensis* leaf extracts is estimated to exceed 5000 mg/kg body weight, indicating a high safety margin. Additionally, mice treated with the extracts showed slight weight gain, suggesting no adverse impact on overall health. Among the tested extracts, the ethyl acetate extract exhibited the highest chemo-suppressive antimalarial activity, achieving 80.7% inhibition of parasite growth in a dose-dependent manner, demonstrating its potential to suppress parasite proliferation during the early stages of infection.
Conclusion: *J. tanjorensis* leaf extracts are promising candidates for further investigation as safe and effective antimalarial therapies, particularly for early-stage infections.

Keywords: Toxicity, Histopathology, Antimalarial Activity, *Plasmodium berghei,* Malaria

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INTRODUCTION

Jatropha tanjorensis, commonly known as “Catholic vegetable” or “Hospital too far”, is a perennial plant belonging to the Euphorbiaceae family that shares phenotypic characteristics with *J. gossypifolia* and *J. curcas* [1]. The plant is widely recognized in Nigeria by various local names—'Iyana-Ipaja' in Yoruba, 'Ugu-Oyibo' in Igbo, and 'Kafi likita' in Hausa—and serves both as a traditional medicine and a dietary staple [2].

The extensive use of *J. tanjorensis* in traditional medicine has been well-documented, with studies highlighting its antimalarial potential. For instance, the aqueous leaf extract of the plant has shown significant antimalarial activity in murine models infected with *Plasmodium berghei,* reducing parasitemia levels and improving hematological parameters without inducing acute toxicity [3]. Its traditional application in treating malaria, anemia, and diabetes further emphasizes the importance of toxicological evaluation to ensure its safety and efficacy [4]. Beyond its antimalarial activity, the plant has demonstrated antimicrobial, hypoglycemic, antihypertensive, antidiabetic, and hematological effects [5,6]. Notably, it exhibits significant antiplasmodial activity against *P. falciparum* and *P. berghei* [7-10]. Despite these promising medicinal properties, much of the nutritional and therapeutic potential of *J. tanjorensis* remains underexplored [11].

Malaria continues to pose a significant global health challenge, particularly in sub-Saharan Africa. According to the World Health Organization (WHO), there were 247 million malaria cases and 619,000 related deaths in 2021, with 95% of cases and 96% of deaths occurring in Africa. Nigeria alone accounted for 31.3% of global malaria deaths and 27% of all malaria cases during this period [12]. In Nigeria, approximately 50% of adults experience at least one

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malaria episode annually, while children under five endure two to four attacks per year, making malaria responsible for 60% of all hospital visits [13]. The growing resistance of *Plasmodium* species to commonly used antimalarial drugs has further exacerbated this public health crisis leading to the introduction of Artemisinin-based combination therapies (ACTs) to combat resistance [14,15]. However, reports of ACT failure in Southeast Asia, particularly in the Greater Mekong Subregion, highlight the urgent need for alternative therapies [16]. Drug resistance is also becoming a concern in Nigeria, with cases of *P. falciparum* showing reduced sensitivity to artemether-lumefantrine [17,18]. For instance, a randomized controlled trial in Mali and Burkina Faso found a higher recurrence of *P. falciparum* parasitemia among patients treated with artemether-lumefantrine than those treated with other regimens [19]. These findings emphasize the potential threat to the continued efficacy of artemether-lumefantrine in West Africa and the pressing need for innovative antimalarial strategies [20,21].

This study aimed to evaluate the acute oral toxicity, histopathological effects, and chemo-suppressive antimalarial potential of *J. tanjorensis* leaf extracts. By rigorously assessing its safety and therapeutic efficacy, this study sought to advance the understanding of *J. tanjorensis* as a potential antimalarial agent, contributing to the development of future malaria treatment strategies.

METHODS

Study design

The experimental research design employed a simple random sampling technique to select and group experimental animals in compliance with Standard Protocols and Good Laboratory Practice (GLP) guidelines. The procedures used in the present study also adhered to the guidelines of the Organization for Economic Co-operation and Development (OECD) [22] and Animal Research Reporting of *In Vivo* Experiments (ARRIVE). A completely randomized design was used to minimize bias and enhance the reliability of the findings.

Ethical approval

Ethical approval for the use of experimental animals and the conduct of this research was granted under approval number ABUCAUC/2023/034 by the Committee on Animal Use and Care, Directorate of Academic Planning and Monitoring, Ahmadu Bello University (A.B.U), Zaria, Kaduna State, Nigeria. This committee ensures adherence to internationally accepted guidelines for the humane treatment of laboratory animals.

Collection and authentication of plant material

Fresh leaves of *J. tanjorensis* (Catholic vegetable) were harvested in February 2023 from private gardens located at 11°04'N, 7°42'E within the Zaria Local Government Area of Kaduna State. A sample of these leaves was taken to the Herbarium unit of the Department of Botany, Ahmadu Bello University (A.B.U), Zaria, and assigned voucher number V/N-ABU01911. A voucher specimen was preserved in the herbarium for reference.

Preparation and extraction of plant material

The study prepared fresh *J. tanjorensis* leaves by washing, drying, pulverizing, and storing them for analysis, following a method described by Aiwonegbe et al. [5]. *J. tanjorensis* leaves were extracted using the maceration technique described by Matabane et al. [23]. The crude extraction involved soaking powdered leaves in methanol for 72 hours, filtering, and concentrating while the sequential extraction involved soaking powdered leaves in three solvents (n-hexane, ethyl acetate, and methanol) for 72 h each, followed by filtration and concentration.

Determination of acute oral toxicity of J. tanjorensis leaf extracts

Swiss albino mice weighing 20–40 g were obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The mice were acclimatized to the laboratory conditions for two weeks under a 12-hour light/dark cycle in a well-ventilated environment. They were housed in clean, dry wood shavings, and provided with standard feed and water [24]. The LD_{50} was determined using the protocol described by Shehu et al. [25] in adherence to the OECD 425 guidelines. Stock solutions of the extracts were prepared by dissolving 1 g of each extract in 2 ml (500 mg/ml) of sterile distilled water. Five mice were randomly selected and given a dose of 5000 mg/kg body weight, and monitored for signs of toxicity, including tremors, convulsions, and death.

Determination of the histopathological impact of J. tanjorensis leaf extracts on kidney and liver sections of mice

Histopathological examinations of liver and kidney tissues were performed following the method described by Najman et al. [26]. After completing the 14-day acute toxicity study, the mice were humanely sacrificed using ketamine anesthesia. The organs were fixed, dehydrated, cleared, and embedded in paraffin wax. Thin sections were prepared and stained, and the tissues were mounted under coverslips. Microscopic examinations focused on identifying pathological changes, such as lesions, due to exposure to test extracts.

Chemo-suppressive potential of *J. tanjorensis* leaf extracts against *Plasmodium berghei*

1. Grouping and dosing of experimental animals: Swiss albino mice were used in an in vivo suppressive test, acclimatized for two weeks, and doses of 400, 200, and 100 mg/kg were tested corresponding to 8, 4, and 2 % of the LD_{50}, respectively. The method described by Misganaw et al. [27] was adopted, involving 70 mice, divided into groups, (I, II, III, IV, V, and VI), as shown in Table 1.

2. Parasite inoculation: *Plasmodium berghei* NK-65 was obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The method described by Ndem et al. [28] was followed, in which the parasite was maintained in three donor mice through serial passaging. Blood (1-2 ml) from donor mice with a parasitemia level of 25-28% was collected via retro-orbital puncture into a tube containing 2% trisodium citrate as an anticoagulant. This blood was diluted in physiological saline to achieve a concentration of 1×10^7 infected red blood cells (RBCs). Each experimental mouse was inoculated intraperitoneally with 0.2 ml of the diluted blood containing 1×10^7 *P. berghei* NK-65 infected erythrocytes.

Table 1. Grouping and dosing of experimental and control mice

Group	Number of mice	Treatment	Graded doses (mg/kg body weight)	Route
I	5	C-MeOH)	100	Oral
I	5	(C-MeOH)	200	Oral
I	5	(C-MeOH)	400	Oral
II	5	(S-nHex)	100	Oral
II	5	(S-nHex)	200	Oral
II	5	(S-nHex)	400	Oral
III	5	(S-Ethyl A)	100	Oral
III	5	(S-Ethyl A)	200	Oral
III	5	(S-Ethyl A)	400	Oral
IV	5	(S-MeOH)	100	Oral
IV	5	(S-MeOH)	200	Oral
IV	5	(S-MeOH)	400	Oral
V	5	(CQ)	5	Oral
VI	5	(DW)	10 ml/kg	Oral

C-MeOH = Crude Methanol extract; S-nHex = Sequential n-hexane extract; S-Ethyl A = Sequential Ethyl acetate extract; S-MeOH = Sequential Methanol extract; CQ: Chloroquine (positive control); DW: Distilled Water (negative control).

3. Chemo-suppressive test: The four-day chemo-suppressive test of *J. tanjorensis* leaf extracts was conducted following the method outlined by Misganaw et al. [27]. After inoculation, all animal groups were treated with the extracts and positive control. Graded doses were administered orally to mice daily for four days. Thin blood films were prepared and examined microscopically to compare the average parasitemia in each test group and the positive control group.

$$\% \text{ Suppression} = \frac{\text{Mean Parasitemia in negative control group} - \text{Mean Parasitemia in test group}}{\text{Mean Parasitemia in the negative control group}} \times 100$$

The mean survival time (MST) for each group was calculated from the date of infection to 30 days. The mean survival time was calculated using the following equation:

$$MST = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in the group}}$$

Data analysis

The study used descriptive and inferential statistics to analyze data from three replicates, with statistical significance set at $p < 0.05$.

RESULTS

Extraction yield of *J. tanjorensis* leaves

The crude methanol extract (C-MeOH) obtained through maceration yielded 20.86 % (20.86 g) per 100 g of plant powder. The sequential maceration process resulted in three extracts: n-hexane (S-nHex), ethyl acetate (S-Ethyl A), and methanol (S-MeOH). Among these, S-MeOH extract had the highest yield of 19.43 % (90.94 g) per 468 g of marc. This was followed by the S-nHex extract, which yielded 2.33 % (11.62 g) per 500 g of plant powder. The S-Ethyl A extract had the lowest yield of 1.94 % (9.42 g) per 485 g of marc.

Lethal dose (LD_{50}) of *J. tanjorensis* leaf extracts by acute toxicity study

By the end of the study, one mouse died in the groups administered S-ethyl A and S-MeOH extracts, while all five mice in the test groups administered C-MeOH and S-nHex extracts survived throughout the study and no signs of acute toxicity were observed in the mice. According to OECD 425 guidelines, LD_{50} is considered greater than 5000 mg/kg when more than three animals survive at the dose of 5000 mg/kg. Thus, the LD_{50} of *J. tanjorensis* leaf extracts was inferred to be >5000 mg/kg, indicating a high safety margin.

Figure 1 presents the microscopic features of the organs harvested from experimental and control mice. The kidney sections of mice administered with sequential ethyl acetate, sequential methanol, and sequential n-hexane extracts exhibited slight histopathological changes, including tubular necrosis and hyperplasia of inflammatory cells. In contrast, the group treated with the crude methanol extract showed normal kidney morphology. In the liver sections, slight Kupffer cell hyperplasia was observed in the group administered the crude methanol extract, whereas the groups treated with sequential ethyl acetate, sequential methanol, and sequential n-hexane extracts showed normal liver features.

Figure 2 shows no significant percentage change in the kidney weight of the experimental mice groups (4.03 – 4.58

Table 2. Acute toxicity response of Swiss albino mice exposed to *J. tanjorensis* leaf extracts

Groups	No. of mice/group	Dose (mg/kg)	Toxicological symptoms	No. of deaths	Mortality (%)
C-MeOH	5	5000	None	0	0
Test S-nHex	5	5000	None	0	0
Test S-Ethyl A	5	5000	None	1	20
Test S-MeOH	5	5000	None	1	20
Control	5	10ml/kg	None	0	0

C-MeOH = Crude Methanol extract; S-nHex = Sequential n-Hexane extract; S-Ethyl A = Sequential Ethyl Acetate extract; S-MeOH = Sequential Methanol extract; Control: Water (Vehicle).

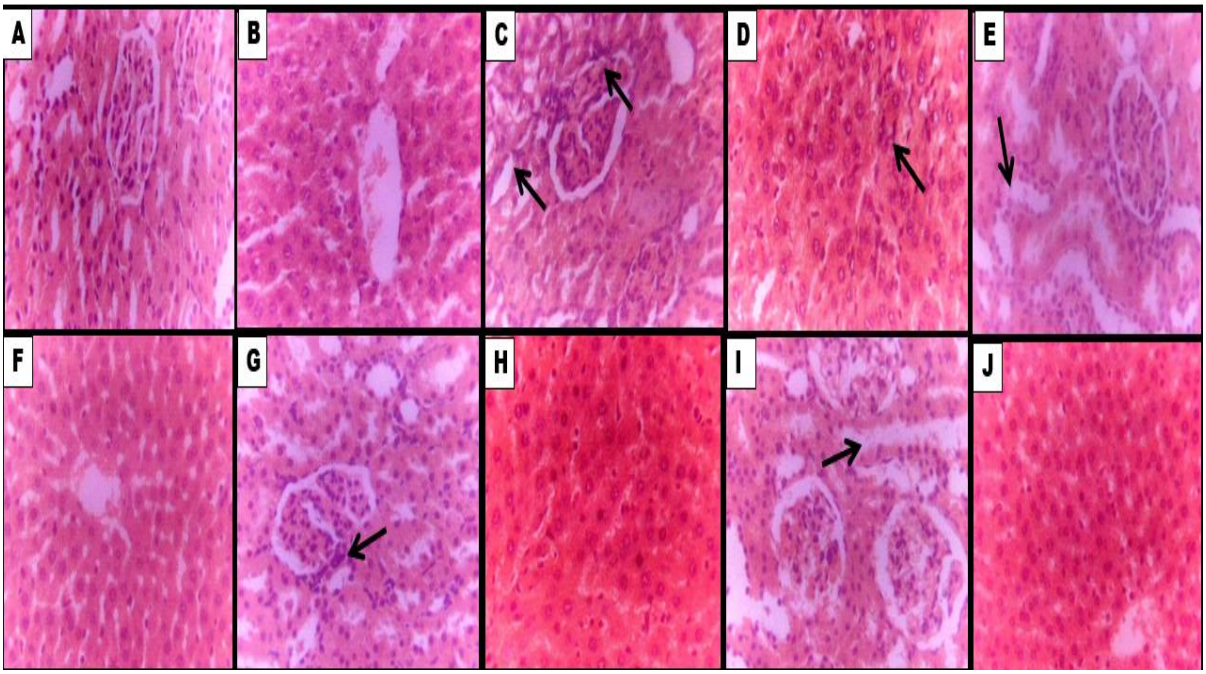

Figure 1. Histopathological changes of the liver and kidney of Swiss albino mice administered extracts of *J. tanjorensis* leaf across the period of acute toxicity study
(A) Control=Kidney section of mice given water; (B) Control=Liver section of mice given water; (C) C-MeOH =Kidney section of mice given crude methanol extract; (D) C-MeOH =Liver section of mice given crude methanol extract; (E) S-nHex =Kidney section of mice given sequential n-hexane extract; (F) S-nHex =Liver section of mice given sequential n-hexane extract; (G) S-Ethyl A =Kidney section of mice given sequential ethyl acetate extract; (H) S-Ethyl A =Liver section of mice given sequential ethyl acetate extract; (I) S-MeOH =Kidney section of mice given sequential methanol extract; (J) S-MeOH =Liver section of mice given sequential methanol extract.

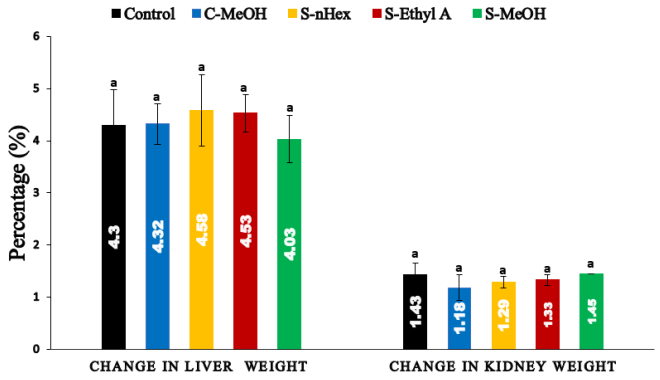

Figure 2. Comparison of percentage change in organ weight of Swiss Albino mice administered extracts of *J. tanjorensis* leaf across the period of acute oral toxicity study
Values are expressed as mean ± SD (n= 3), and values with the same superscript indicate no significant difference across the groups (p>0.05). C-MeOH = Crude Methanol extract; S-nHex = Sequential n-Hexane extract; S-Ethyl A = Sequential Ethyl acetate extract; S-MeOH = Sequential Methanol extract; Control: Water (Vehicle).

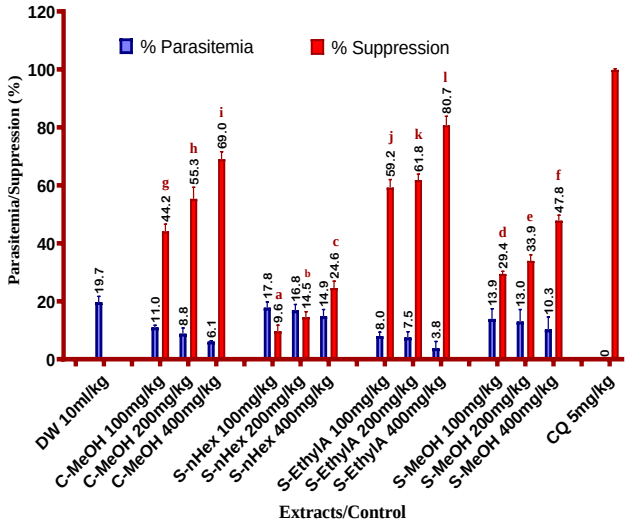

Figure 3. Suppressive potential of *J. tanjorensis* leaf extracts against *Plasmodium berghei*
Values are expressed as mean ± SD (n= 6). Two-way analysis of variance (ANOVA) results were separated using Tukey's post hoc test; values with different superscripts are significantly different at (p <0.05). C-MeOH = Crude Methanol extract; S-nHex = Sequential n-hexane extract; S-Ethyl A = Sequential Ethyl acetate extract; S-MeOH = Sequential Methanol extract; CQ: Chloroquine (positive control); DW: Distilled Water (negative control).

%) compared to the control mice group (4.3 %) (P>0.05). There was also no significant percentage change in the liver weight of the mice in the experimental groups (1.18 – 1.45 %) compared to that in the control mice group (1.43 %) (P>0.05).

The sequential ethyl acetate extract showed the highest suppression percentage compared to the positive control (p < 0.05), but it remained comparable. It also showed dose-dependent suppression, with no significant difference between 100 mg/kg and 200 mg/kg doses (p = 0.4268).

Table 3 shows the mean body weight changes and survival time of Swiss albino mice infected with *P. berghei* and treated with *J. tanjorensis* leaf extracts or the control. Results showed significant differences in percentage body weight change and

Table 3. Mean body weight changes and mean survival time of Swiss Albino mice infected with *Plasmodium berghei* and treated with *J. tanjorensis* leaf extracts and control across the period of suppressive test

Groups	Dose (mg/kg)	Mean Body Weight (D0)	Mean Body Weight (D5)	% Change	Mean Survival Time (Days)
Positive Control (CQ)	5	23.40±2.79	27.60±2.07	17.95^{n}	30.00±0.00^{c}
Negative control (DW)	10	25.60±1.04	22.60±1.78	-11.72 ^{a}	28.80±2.68^{a}
C-MeOH	100	23.80±2.05	26.80±2.42	12.61^{l}	30.00±0.00^{c}
C-MeOH	200	23.00±1.16	26.60±1.61	15.65^{m}	30.00±0.00^{c}
C-MeOH	400	21.00±1.92	25.80±1.30	22.86^{o}	30.00±0.00^{c}
S-nHex	100	23.80±0.70	23.40±0.98	-1.68^{b}	29.00±1.41^{b}
S-nHex	200	26.20±2.27	26.00±2.74	-0.76^{c}	30.00±0.00^{c}
S-nHex	400	24.80±2.59	25.20±2.61	1.61^{e}	30.00±0.00^{c}
S-Ethyl A	100	22.80±2.68	23.60±2.30	3.23^{g}	30.00±0.00^{c}
S-Ethyl A	200	24.00±2.55	25.20±2.86	5.00^{h}	30.00±0.00^{c}
S-Ethyl A	400	24.20±1.30	26.40±1.14	9.09^{k}	30.00±0.00^{c}
S-MeOH	100	22.60±1.82	23.20±1.79	2.65^{f}	29.40±1.34b^{c}
S-MeOH	200	21.40±0.55	22.60±0.89	5.61^{hi}	30.00±0.00^{c}
S-MeOH	400	24.00±0.54	26.10±0.39	8.75^{j}	30.00±0.00^{c}

Values are expressed as the mean ± SD (n= 6), and values with different superscripts are significantly different at (p <0.05). C-MeOH = Crude Methanol extract; S-nHex = Sequential n-Hexane extract; S-Ethyl A = Sequential Ethyl acetate extract; S-MeOH = Sequential Methanol extract; CQ: Chloroquine (positive control); DW: Distilled Water (negative control).

survival time between experimental groups, with C-MeOH extract showing the highest percentage increase ($p < 0.05$).

DISCUSSION

The present study determined that the lethal dose (LD_{50}) of *J. tanjorensis* leaf extracts exceeded 5000 mg/kg, indicating that the extracts are non-toxic and safe for oral administration. These findings are consistent with previous studies by Anhwange et al. [3], Aiwonegbe et al. [5], Babayemi et al. [29], and Ezendekwere et al. [30]. Furthermore, the observed LD_{50} of >5000 mg/kg aligns with the findings of Umoren et al. [31], who reported the safety of *J. tanjorensis* extracts at doses up to 8000 mg/kg body weight in experimental animals.

The histological findings indicated no gross pathological changes in the organs following extract exposure. These observations are in agreement with those of Chibuogwu et al. [32], who reported mild vacuolar degeneration and necrosis of hepatocytes after an acute toxicity study. The mild toxic effects observed in this study could be attributed to tannic acid exposure from different levels of tannins present in the extracts [33].

Considering the oral administration safety of *J. tanjorensis* leaf extracts established in this study with an LD_{50} > 5000 mg/kg, as per OECD guideline No. 425 (2008), this study further evaluated the *in vivo* antiplasmodial activity of the extracts against *P. berghei* using a suppressive test. The *in vivo* study was essential to account for potential prodrug effects and the role of the immune system in clearing the infection. *P. berghei*-infected Swiss albino mice were used as experimental models for human malaria caused by *P. falciparum* because of similarities in pathophysiology, molecular biology, and mode of disease transmission between the two species [34].

The findings revealed that sequential ethyl acetate extract exhibited the highest chemo-suppressive activity in a dose-dependent manner, reducing parasitemia by 80.7% at a dose of 400 mg/kg. This performance was comparable to that of the positive control group treated with chloroquine, which achieved a 100% parasitemia reduction. However, unlike refined chloroquine, the plant extracts were tested in crude form. These findings agree with those of Ebenyi et al. [35], who reported the antiplasmodial effect of the aqueous leaf extract of *J. tanjorensis* on parasitemia and hematological parameters in *P. berghei*-infected mice. This suggests that *J. tanjorensis* leaves possess the potential to inhibit malarial parasite growth in the early stages of infection, offering prospects for disease control.

CONCLUSION

The crude extracts of *J. tanjorensis* leaves exhibited an LD_{50} greater than 5000 mg/kg, with no gross pathological changes observed in the organ sections of mice exposed to the extracts, indicating their safety for oral administration and their potential therapeutic use. Furthermore, the ethyl acetate extract demonstrated an 80.7% suppressive effect in *vivo*, accompanied by weight gain in experimental mice. These findings align with broader research on plant-based antimalarial agents, demonstrating the therapeutic potential of *J. tanjorensis*, both safe and effective. The antimalarial activity of *J. tanjorensis* may be attributed to bioactive flavonoids, alkaloids, and phenolic compounds, which are known for their antimalarial and immunomodulatory properties as reported in previous phytochemical studies.

However, the exact mechanisms of action, potentially involving interference with the life cycle of the malarial parasite or disruption of redox pathways, require further investigation.

LIMITATIONS

The lack of information on the long-term effects of *J. tanjorensis* leaf extracts in experimental mice limited this study. However, the short-term impact and demonstrated suppressive antimalarial effects provide a solid foundation for collaborative efforts among researchers in microbiology, pharmaceutical sciences, clinical fields, and the pharmaceutical industry to explore and fully harness the therapeutic potential of this plant.

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