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Original Research Article

Antiplasmodial and cytotoxic activities of *Jussiaea linifolia* G. Don extracts

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Abstract

Purpose: *To evaluate the in vivo and in vitro antiplasmodial activity of Jussiaea linifolia extracts using validated models.*
Methods: *Methanol extract was partitioned into n-hexane, ethyl acetate and n-butanol fractions and subjected to antiplasmodial and cytotoxic activity assays. The in vivo assay adopted Peter's four-day suppressive and Ranes curative tests to estimate Plasmodium berghei NK47 growth suppression while the in vitro antiplasmodial activities were performed using chloroquine-sensitive Plasmodium falciparum NF54 and L6 mammalian myoblast to determine growth inhibition and cytotoxicity respectively.*
Results: *Acute toxicity test showed that the methanol extract displayed LD_{50} > 5000 mg/kg. The in vitro assays revealed that the extract and ethyl acetate fraction elicited significantly higher IC_{50} of 1.15 µg/mL (L6 83.2 µg/mL) and 0.785 µg/mL (L6 > 100 µg/mL), respectively against P. falciparum compared with n-hexane (> 100 µg/mL; L6 5.89 µg/mL) and n-butanol (48.1 µg/mL; L6 12.84 µg/mL) fractions. In the in vivo suppressive model, 400 mg/kg of ethyl acetate soluble fraction elicited a 97.1 % ($p < 0.05$; mean survival time > 21 days) P. berghei suppression compared with untreated group. Also, the ethyl acetate soluble evoked the highest suppression of parasitemia (94.17 %) in the curative model when compared with untreated. The extract and fractions of J. linifolia were found to restore packed cell volume in infected mice to their respective baselines compared with continued decline in untreated group.*
Conclusion: *The study validates the traditional use of J. linifolia as an antimalarial decoction in some rural communities and shows that the ethyl acetate soluble fraction of methanol extract could be a source of lead antiplasmodial compounds.*

Keywords: *Antiplasmodial, Curative, Ethnomedicine, Jussiaea linifolia, Phytochemicals, Suppressive*

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INTRODUCTION

The medicinal use of herbs and herbal products in traditional medicines has sustained the healthcare economies of many countries [1]. Apart from being the source of lead compounds, plants are readily accessible and relatively safe in management of common health challenges. Their potency is largely attributed to the synergism of phytochemical constituents, which is the basis of polypharmacology [2]. These herbs, including *Jussiaea linifolia* Vahl of the Onagraceae family, are well distributed in tropical regions. *Jussiaea linifolia* (synonymous with *Jussiaea hyssopifolia* G. Don and *Ludwigia hyssopifolia* G. Don) Exell), also known as water

primrose, is an herbaceous, aquatic, or marshy plant with diverse ethnomedicinal importance [3]. In traditional medicine, *J. linifolia* is used in the treatment of many parasitic infections [4]. Some of these uses have been attributed to the phytochemical content of the plant [5].

Among the Wanpie Fadama settlement in Zaki-Biam, North Central Nigeria, a decoction of the aerial part of the plant is used in ameliorating the feverish conditions associated with malaria, a parasitic infection that causes over 600,000 mortalities in sub-Saharan Africa annually [6-8]. To date, Sub-Saharan Africa has remained the epicenter of malaria infection, posing a great threat to socioeconomic and health development [9,10]. Transmission is reduced by the human immune system, however, treatment of *P. falciparum* malaria is still hampered by the emergence of multi-drug resistant strains of *P. falciparum*, cost of treatment and adverse drug effects [11].

In addition to various control measures, chemotherapy has remained the mainstay in the prevention and treatment of malaria. However, constant treatment of malaria with already existing antimalarial drugs has posed a fast spread of multi-drug resistant *Plasmodium* parasites [7]. Consequently, the development of resistance to various insecticides and the lack of an effective vaccine in adults constitute challenges in preventing and controlling malaria, thus increasing hospitalization and mortality rates [8]. Early discovery of quinine from *Cinchona species* and artemisinin from *Artemisia annua* supports a strong historical link between malaria chemotherapy and natural products [8]. One of the advantages of herbal remedies in this regard is the synergistic potential of polyphytochemical constituents of the herbs via multiple target inhibition of relevant proteins and enzymes in the parasite [10]. Although there are several researches on the ethnopharmacological relevance of *J. linifolia*, the claimed use of decoction of the aerial part of the plant as an antimalarial remedy in folk medicine by the Wanpie Fadama people or any other tribe has not been validated. The study, therefore, investigated the antiplasmodial and cytotoxic activities of aerial parts of *J. linifolia*.

EXPERIMENTAL

Plant material

The aerial parts of *J. linifolia* were harvested in November 2022, in Zaki-Biam, North Central Nigeria and were identified and authenticated by a Taxonomist, Mr Felix Nwafor, of the Department of Plant Science and a specimen (voucher ID: PCG/UNN/0417) deposited at the Herbarium of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. The plant name was further confirmed at https://www.theplantlist.org on the 16th of January, 2023. The aerial parts of *J. linifolia* were shade-dried at 25 ºC for 4 weeks and the dried sample was pulverized into a coarse powder using a mechanised grinding machine.

Mice

Healthy Swiss albino mice (6 – 10 weeks old; 32.5 ± 2.5 g) of either sex were used for the acute toxicity and antiplasmodial activity assays. The mice were bred at the Animal House of the Veterinary Teaching Hospital of University of Nigeria Nsukka, housed in well-ventilated cages at 25 °C and fed with a pellet diet and water *ad libitum*. They were acclimatised for 7 days before the start of treatments. The protocol for the use of mice in this study was approved by the Committee on Animal Ethics of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (reference no. FPSRE/UNN/22/0002). Mice were handled by following the guidelines on the care and use of laboratory animals [12].

Parasite

A culture of chloroquine-sensitive *Plasmodium berghei* (NK47 strain) was gifted by the Veterinary Teaching Hospital, University of Nigeria, Nsukka. The cold-preserved *P. berghei* was injected (200 µL containing 10^6 parasites) into a recipient mice intraperitoneally (i.p.). On the 4th day post-infection, parasitemia in the blood obtained from the tail vein was monitored daily using a Giemsa-stained thin smear under oil immersion microscopy. Passage of parasites in new mice consisted of an i.p. injection of 200 µL of 10^6 infected erythrocytes [7,8]. *Plasmodium falciparum* (intraerythrocytic form, NF54 IEF strains) and L6 rat skeletal myoblast were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with Albumax (~5 %), L-glutamine (2 mM), hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES; 5.95 g/L), sodium bicarbonate (2 g/L) and 10 % fetal bovine serum.

Preparation of plant extract

The coarsely powdered aerial parts of *J. linifolia* (500 g) were macerated successively in 5.0 L (2 x 2.5 L) methanol (96 % v/v) for 48 h with intermittent agitation. The mixture was filtered using a Whatman filter paper No.1 and the filtrate

was concentrated under vacuum to obtain *J. linifolia* methanol extract (JME). This was stored for further use.

Partitioning of extract in different solvents

A 10 g of JME was dispersed in 200 mL of aqueous methanol (10 % v/v) using a high-speed magnetic stirrer for 10 min [13]. The dispersion was transferred to a separation funnel, made up to 500 mL and partitioned successively with 500 mL each of *n*-hexane, ethyl acetate and *n*-butanol, each solvent partition repeated until the clear solvent was obtained. The fractions were concentrated to dryness under a vacuum to obtain the *n*-hexane (JHF), ethyl acetate (JEF) and *n*-butanol (JBF) fractions of JME.

Phytochemical screening

The presence or otherwise of major secondary metabolites in JME, JHF, JEF and JBF were tested following standard protocols [14]. The metabolites tested for included alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, anthraquinones and steroids.

Acute toxicity test

A modified Lorke's method was used to determine the acute toxicity (LD_{50}) of JME [14] and the two phases involved thirteen mice. The first step involved the use of nine mice, divided into three groups (n = 3) and a control. Each group received 10, 100 and 1000 mg of JME per kg of the mouse, respectively, while the control mouse received the blank. The mice were then monitored for gross behavoiral changes. In the second phase, three groups (n = 1) received 2000, 3500 and 5000 mg JME per kg of mouse, respectively, since no death of mice was recorded in phase one. The LD_{50} was calculated by the geometric mean of the minimum toxic and the maximum tolerated doses of JME.

***In vitro* antiprotozoal and cytotoxicity assays**

The *in vitro* antiprotozoal screening of the extract and fractions against the intraerythrocytic form of *P. falciparum* (NF54 IEF strain) and cytotoxicity test against L6 cell line from rat skeletal myoblasts was performed according to established standard protocol using chloroquine and podophylotoxin as controls [9]. Serial dilutions of the controls, JME and fractions were prepared in the 100, 200 and 100 µL growth media from the top concentration of 0.10, 400 and 200 µg/mL concentration of controls, JME and fraction respectively and diluted accordingly. The culture and test samples were incubated for 48 h at 37 ºC in a 5 % CO_2 atmosphere.

Induction of parasitemia and inoculation of mice

Plasmodium berghei-infected mouse (containing 20 to 30 % parasitemia) was employed as a donor [7]. Infected blood was obtained from the donor via cardiac puncture into a heparinized capillary tube containing 0.5 % trisodium citrate which was then diluted to 5 x 10^7 cells/mL with normal saline (0.9 % v/v). Experimental mice were inoculated with 0.2 mL blood suspension (i.p.) containing 1.0 × 10^8 *P. berghei*-parasitized erythrocytes on the first day (day 1) and were closely observed for 2 h before randomly grouping the mice.

Grouping and dosing of mice

The dosing was selected based on the result of the acute toxicity test. Therapeutic doses between the range of 0.10 and 0.01 of the calculated LD_{50} (> 5000 mg/kg) were selected for administration. The mice were randomly divided into nine groups (n = 5) for each of the suppressive and curative tests (Table 1).

Table 1: Grouping and dosing of mice for antiplasmodial activity

Group	Suppressive/curative test (mg/kg BW)
A	Infected and treated with 7 mg of AL
B	Infected and treated with 100 mg of JME
C	Infected and treated with 200 mg of JME
D	Infected and treated with 400 mg of JME
E	Infected and treated with 100 mg of JEF
F	Infected. and treated with 200 mg of JEF
G	Infected and treated with 400 mg of JEF
H	Infected but not treated
I	Neither infected nor treated

Artemeter/lumenfanthrine combination (AL)

***In vivo* antiplasmodial activity screening**

The *in vivo* antiplasmodial activity of *J. linifolia* was evaluated using Peter's four-day suppressive and Ranes curative tests to determine parasitemia and percentage suppressions [8]. In both models, baseline values for all parameters such as rectal temperature, body weight and hematological and biochemical parameters were established just before infection and treatment.

Peter's four-day suppressive test

To evaluate the schizontocidal activity of *J. linifolia* extract and fractions [8], treatment (i.p.) commenced 3 h post-inoculation with the

parasite on the first day (day 1) and continued daily for four days (day 4) as indicated in Table 1.

On the 4th day, 1 mL of blood was collected from the mice under anesthesia by cardiac puncture and divided in equal volumes into plain and heparinized microtubes for determination of *P. berghei* growth suppression. The heparinized and non-heparinized blood samples were used for the determination of hematological and biochemical parameters, respectively.

Curative (Ranes) test

To evaluate the effect of *J. linifolia* extract and fractions on an established infection, 1 mL of blood was collected from the tails of mice in each group on the 4th day (day 4) post-inoculation to prepare a thin blood film for the determination of parasitemia.

The treatments (single daily oral dose) commenced thereafter and continued daily for four days (days 4 to 7). On the 7th day post-inoculation, the levels of parasitemia were determined for each group and the percentage curative was calculated [8].

Estimation of level of parasitemia

A thin blood film was prepared on microscopic slides, fixed in MeOH and stained with Giemsa dye (10 %, pH 7.2) for 15 min. The parasitized red blood cells (RBCs) were counted out of 100 RBCs in each field under a light microscope in 10 random microscopic fields [8]. The mean parasitemia from the 10 randomly selected fields was calculated and the percentage suppression (S) was evaluated using Eq 1.

$$S (\%) = \{(PNC–PT)/PNC\}100 \quad \text{.....(1)}$$

Where PNC = Parasitaemia of negative control; PT = Parasitaemia of treated

Determination of survival time

Each mouse in the curative test model was observed daily to determine the survival time of the mice. The mean survival time (MST) was estimated for 28 days starting from day 1 [8]. The MST in each group was computed using Eq 2.

$$MST = \sum SD/\sum NM \quad \text{..... (2)}$$

Where $\sum SD$ = Sum of survival days of all mice in a group, $\sum NM$ = Sum of number of mice per group

Determination of haematological and biochemical parameters

Blood samples collected in non-heparinized microtubes were centrifuged for 10 min at 4000 rpm. The serum obtained was used for determination of transaminase enzymes such as the aspartate (AST) and alanine aminotransferases (ALT).

Samples in heparinized microtubes were used in the determination of hematological indices such as RBC count, packed cell volume (PCV) and hemoglobin (Hb) using an auto-hematological analyzer [8].

Determination of body weight and rectal temperature

In the suppressive and curative tests, the weight and rectal temperature of each mouse were determined pre-infection, 4 h post-infection and then daily for 5 days [8].

Data analysis

Data were expressed as mean ± standard error of the mean (SEM). Control groups were compared with treatment groups using one-way ANOVA followed by Dunett's posthoc analysis using GraphPad Prism v.5. Results were considered statistically significant at $p < 0.05$.

RESULTS

Extraction yield and fractionation of JME

The cold methanol extraction of *J. linifolia* aerial parts yielded a dark brown mass, JME, of 54.25 g representing 10.8 % (w/w) of dried coarse powder. On further separation by a solvent fraction, 10 g of JME yielded 3.5175 g of JMF, 2.70 g of JEF and 2.5025 g of JHF, representing 3.5, 2.7 and 2.5 % (w/w) of dried coarse powder.

Phytochemical composition of *J. linifolia*

The phytochemical constituents of JME and its fractions were determined by standard methods. The crude extract, JME, contained alkaloids, flavonoids and tannins in high concentration while anthraquinones and cardiac glycosides were detected in trace quantities.

On fractionation, alkaloids and flavonoids were detected in high concentrations in both JBF and JEF while steroids and terpenoids were partitioned in the JHF (Table 2).

Table 2: Phytochemical constituents of *J. linifolia*

Secondary metabolites	JME	JBF	JEF	JHF
Alkaloids	+	+	+	-
Anthraquinones	+	+	-	-
Cardiac glycosides	+	+	-	-
Flavonoids	+	+	+	-
Saponins	+	+	-	-
Steroids	+	-	-	+
Tannins	+	+	-	-
Terpenoids	+	-	-	+

(+) = present; (-) = absent

Acute toxicity of JME

The JME did not show any sign of acute toxicity or death on the administration of 5000 mg of JME per kg of mouse. There were also no toxicity-related changes in the gross behaviour of mice such as involuntary and feeding activities.

Antiplasmodial activity of *J. linifolia*

In vitro antiplasmodial and cytotoxic activities

The extract and its fractions showed varying *in vitro* antiplasmodial and cytotoxic activities against *P. falciparum* and mammalian L6 cells, respectively (Table 3). The extract, with a moderate IC_{50} of 1.15 µg/mL, showed a significant change in the activity against *P. falciparum* on partitioning. Unlike the JBF and JHF, there was an improvement in the activity of JEF compared with JME, with no corresponding effect on the mammalian L6 cells. The most active sample, JEF, appeared to be the least cytotoxic, with IC_{50} of > 100 µg/mL. However, JME and its fractions showed significantly ($p < 0.05$) lower activity against Pfc compared with the standard.

In vivo suppressive effect of J. linifolia extract and fractions

All administered doses of JME elicited a significant decline ($p < 0.05$) in the percentage of parasitemia when compared with untreated control mice (group H) and also extended the mean survival time (MST) of mice by 12 to 15 days (Table 4). The results also showed that JEF elicited the strongest parasite suppression (> 90 %) when compared with other treatments. There was no significant difference ($p > 0.05$) in the suppression of *P. berghei* at all doses of JEF when compared with the standard. However, JHF showed the least suppression among the fractions with 200 mg/kg causing a 45.6 % suppression of *P. berghei*.

Table 3: *In vitro* antiplasmodial and cytotoxic activities of *J. linifolia*

Sample	Mean IC_{50} (µg/mL)	
	Pfc (NF54)	Cytotox (L6)
JME	1.150±0.034*	83.20±26.83**
JBF	46.084±2.950*	12.84±0.97**
JEF	0.785±0.011*	>100.0
JHF	>100.0	5.89±1.02**
CQN	0.002±0.0	-
PPT	-	0.008±0.001

Pfc (*P. falciparum*), chloroquine (CQN), podophyllotoxin (PPT); *$p < 0.05$ compared to CQN, **$p < 0.05$ compared to PPT

In vivo curative effect of J. linifolia extract and fractions

The curative effect of *J. linifolia* on an established *P. berghei* infection is shown in Table 5. The methanol extract of *J. linifolia* (400 mg/kg) elicited 47.6 % *P. berghei* clearance, significantly lower ($p < 0.05$) than the standard control and extended MST of mice to 16 days. All treatments extended the MST of experimental mice. Except for JEF, other doses of JBF and JHF caused a lower clearance of the parasite. All tested doses of JEF caused a significant decline ($p < 0.05$) in parasitemia and parasite clearance of > 85 % when compared with the untreated control with MST of 20 to 21 days.

Effect of *T. linifolia* on biochemical and hematological indices

The effect of *J. linifolia* on hematological parameters is shown in Figure 1 A. *Plasmodium berghei* infection elicited a significant decline ($p < 0.05$) in PCV, RBC and Hb by 41.0, 49.5 and 30.3 %, respectively in group H when compared to normal group I. Except for JEF-treated mice, there was a dose-dependent increase in PCV compared to group H. However, only the treatment with JEF caused a dose-dependent increase in RBC. Treatments with 200 and 400 mg/kg of JEF caused a significant ($p < 0.05$) increase in Hb compared to the untreated control. No apparent differences were observed among the doses of extract and fractions in protecting the PCV of infected mice.

Also, the effects of *J. linifolia* on the biochemical parameters of *P. berghei*-infected mice are summarized in Figure 1 B. *Plasmodium berghei* infection elicited a significant increase ($p < 0.05$) in the AST and ALT by 13.6 and 82.9 %, respectively, in group H when compared to normal mice in group I.

Table 4: Suppressive effect of *J. linifolia* on *P. berghei*-infected mice

Groups	Parasitaemia (%)	Suppression (%)	MST (days)
A	0.33±0.15[a]	96.95±6.62[a]	21.40±5.18
B	5.84±1.25[b]	46.02±9.43[b]	11.40±1.90
C	6.02±2.18[b]	44.36±3.78[b]	12.05±2.87
D	4.82±1.98[b]	55.45±4.32[b]	14.60±3.48
E	1.02±0.21[a]	90.57±8.64[a]	18.43±8.80
F	0.84±0.10[a]	92.24±3.45[a]	20.80±3.29
G	0.31±0.13[a]	97.13±1.4[a]	21.60±4.57
H	10.82±1.32[b]	0.00±0.00	9.80±4.46
I	-	-	-

[a]$p < 0.05$ compared to group A, [b]$p < 0.05$ compared to group F

Table 5: Curative effect of *J. linifolia* on *P. berghei*-infected mice

Group	Parasitaemia (%)		Clearance (%)	MST (days)
	Day 4	Day 7		
A	9.60±1.01	0.36±0.19	97.88±2.89[a]	22.20±4.73
B	9.20±1.09	6.32±1.01	31.30±2.07[b]	14.02±2.90
C	9.34±2.20	5.78±1.45	38.11±3.98[b]	16.05±2.03
D	10.02±0.94	5.25±2.04	47.60±6.32[a,b]	15.98±3.92
E	10.23±1.95	1.45±0.06	85.83±9.94[a]	19,02±2.50
F	9.89±0.98	0.66±0.13	93.33±5.82[a]	21.20±4.18
G	9.09±1.19	0.53±0.04	94.17±4.03[a]	20.80±3.85
H	10.50±1.04	16.95±2.34	0.00±0.00	10.60±1.96
I	-	-	-	-

[a]$p < 0.05$ compared to Group A, [b]$p < 0.05$ compared to Group H

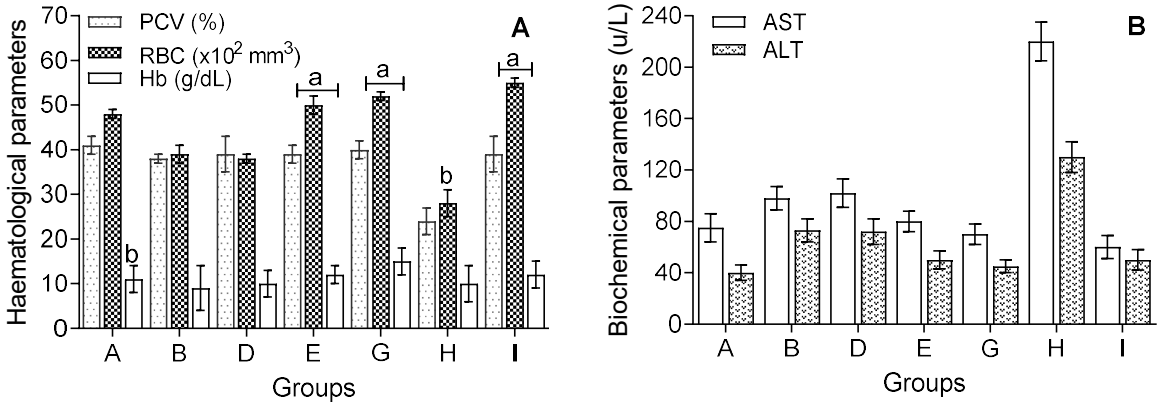

Figure 1: Effect of *J. linifolia* on hematological (A) and biochemical (B) parameters of *P. berghei*-infected mice. [a]$p < 0.05$ compared to group A, [b]$p < 0.05$ compared to group H

Other treatments caused different effects on the liver enzyme activities. Treatments of parasitized mice with JME and its fractions significantly ($p < 0.05$) reduced AST levels. Furthermore, the decline in AST and ALT was statistically significant ($p < 0.05$) in all treatments when compared to both uninfected and untreated (Group I) and the untreated groups (Group H), respectively.

Effect of *T. linifolia* on body weight and rectal temperature

The effect of JME and the most active fraction (JEF) on the body weight of infected mice is shown in Figure 2 A. The treated groups (JME and JEF) and AL showed significant ($p < 0.05$) protection against body weight loss compared to the negative control. The 100 mg JME showed no protection against parasite-induced weight loss, unlike 200 and 400 mg/kg JME and JEF doses, which reversed the initial weight loss. In addition, treatments showed a more significant temperature reduction ($p < 0.05$) when compared with untreated group. Furthermore, JME and JEF protected the treated mice from parasite-induced fever in suppressive model and reversed high temperatures in curative model (Figure 2 B). Consequently, temperature was maintained at < 38.5 °C in all treatment cases compared with > 39.0 °C in the untreated mice.

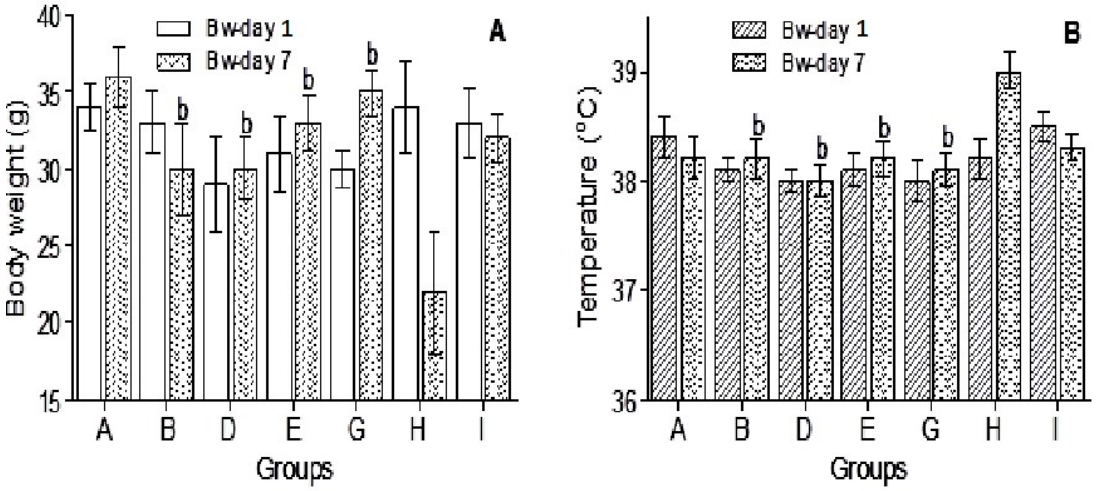

Figure 2: Effect of *J. linifolia* on the body weight (A) and temperature (B) of *P. berghei*-infected mice. $^{b}p < 0.05$ compared to group F, $^{a}p < 0.05$ compared to group A

DISCUSSION

The roles of herbs, including *J. linifolia*, in the management of parasitic infections have continued to increase due to the phytochemical and pharmacological diversity of plants, which complement other activities of *J. linifolia* [3]. Extraction solvents play a vital role in herbal medicines as majority of them are prepared for consumption or application in suitable solvents such as decoction and tincture. Fractionation solvents determine relative abundance and distribution of phytochemical constituents in different solvents [1,9]. The results show that *J. linifolia* constituents have a uniform distribution of polar, moderately polar and non-polar phytochemicals in methanol, ethyl acetate and *n*-hexane, respectively. The preferential partitioning of alkaloids and flavonoids in the JEF, as well as steroids and terpenoids in JHF strongly established the link between the nature and concentration of secondary metabolites and pharmacological activities [1,3].

Several pharmacological activities, including antiplasmodial activity, have been attributed to alkaloids, flavonoids, tannins and phenolic compounds, which are present in relatively high abundance in *J. linifolia* extract and fractions. These phytochemicals are known to be biologically active and could be relatively safe when consumed as medicinal herbs [2,13]. Toxicological studies are an important step in herbal drug discovery as early-stage detection of adverse events caused by the potential drug prevents attrition of candidate drugs at later stage of development [15]. This is also necessary as majority of Africans still rely on herbal medicines for their healthcare needs. No adverse effect was observed even at > 5000 mg/kg of JME, and the absence of physical and behavioural changes observed in this study are good indicators for further studies. It was based on the LD_{50} > 4000 mg/kg that the therapeutic doses of 100 – 400 mg of JME per kg body weight of mouse were selected for antiplasmodial activity study.

The *in vitro* antiplasmodial activity showed that the extract elicited moderate activity against *P. falciparum* NF54 (IC_{50} 1.15 µg/mL) and low cytotoxicity against mammalian L6 cells (IC_{50} 83.20 µg/mL). On partitioning of the extract in solvents of different polarities, there was an improvement in the antiplasmodial activity of JEF and loss of activity of JHF and JBF. The enhanced *in vitro* antiplasmodial activity of JEF corresponds to a decrease in the mammalian L6 cytotoxicity. The JEF represents the midpolar portion of JME and was found to contain alkaloids and flavonoids, which have been reported to possess antiplasmodial activity [16,17]. However, *in vitro* activity does not usually translate to *in vivo* activity in some cases due to the effect of physiological conditions in *in vivo* models [7]. To establish any correlation between the *in vitro* and *in vivo* data, the antiplasmodial activity of JME and JEF was tested in early and established Plasmodium infection models.

The antiplasmodial activities of extract and fractions of *J. linifolia* against *P. berghei* infection in early and established parasitemia were evaluated using the suppressive and curative test models, respectively. The extract elicited significant blood and tissue schizonticidal effects on *P. berghei* at the treatment doses and

prolonged MST beyond 20 and 22 days in the respective models. The schizonticidal effects and MST were even higher in the mice treated with JEF (moderately polar fraction) at the same doses compared with the standard, suggesting that antiplasmodial compounds of *J. linifolia* might be mid-polar. The schizonticidal potential of the plant in early infection provides a chemosuppressive target for the control of malaria infection during early primary invasion of erythrocytes by *P. berghei* [8,11]. Several known schizonticidal antimalarial agents cause clearance or suppression of *Plasmodium* parasites via different mechanisms such as the inhibition of plasmodial dihydrofolate reductase enzyme by pyrimethamine, a tissue schizonticide [10].

The higher suppression of *P. berghei* parasites by JEF showed that the moderately polar constituents of *J. linifolia* such as the alkaloids, glycosides, flavonoids and other phenolic compounds could be antiplasmodial in this study. A pairwise comparison of the effects of JEF and other fractions in both suppressive and curative models reveals interesting insights. The 400 mg/kg dose of JEF consistently elicited > 97 % parasite suppression and clearance in both models. However, there was a lower clearance of parasites in an established infection than in early infection. Derivatives of artemisinin alkylate inhibit *Plasmodium* proteins and heme leading to inhibition of calcium ion transporting ATPase while chloroquine inhibits polymerization of hemozoin by preventing its formation [18]. These and other mechanisms could be responsible for the > 70 % *P. berghei* clearance and suppression observed in the mice treated with 200 and 400 mg/kg of JME and JEF in this study. This, however, is still speculative and further studies that are beyond the present scope are needed to ascertain this.

Plasmodium species are intraerythrocytic parasites that cause a decline in hematocrit and hemoglobin through the rupture of RBC [8]. The rupture is evident in the significant decline in the PCV, RBC and Hb of untreated mice (group H). The decline was, however, reversed to different extents by the various treatments indicating significant RBC protection by the JME and JEF compared to the controls. As indicated by previous researchers, the PCV was assessed to evaluate the effectiveness of *J. linifolia* in preventing hemolysis caused by Plasmodium in RBCs [8]. Anaemia results from the destruction of RBCs and inhibition of erythropoiesis by the parasite. Consequently, PCV analysis is crucial in determining the efficacy of treatments in preventing hemolysis [10,11]. Plants with antimalarial properties are anticipated to prevent a reduction in PCV by mitigating hemolysis. Notably, *J. linifolia* used in both models prevented and reversed *P. berghei*-induced PCV reduction, potentially due to their antiplasmodial effects on parasitized RBCs produced in the bone marrow.

Treatments with JME and JEF reduced significantly the serum ALT and AST load. These tissue-specific liver enzymes are released into the serum when the liver is compromised [19]. Their reduced activities suggest that treatments protected liver integrity or reversed the plasmodium-induced liver damage. Inhibition of viability of *P. berghei* by *J. linifolia* was supported by normalization of weight and body temperature of treated mice. In this study, *P. berghei*-infected mice manifested weight loss and pyrexia. However, JME and its fraction prevented weight loss and fever associated with *Plasmodium* compared to untreated mice. The effects were considerably superior in mice treated with JME and JEF. It is known that rodents infected with *Plasmodium* parasites exhibit fever and considerable weight loss. Furthermore, the decrease of rectal temperatures in treated mice to the baseline values was a further indication of the antiplasmodial activity of *J. linifolia*. It is imperative to point out that this study could not identify or quantify the exact secondary metabolites responsible for the antiplasmodial activity. However, there is a strong link between the phytochemical composition and antiplasmodial activity [10,19,20]. Alkaloids and flavonoids, the major content of the JEF, have been reported to show antiplasmodial activity and most of the conventional antimalarial drugs in use today are primarily alkaloids [21]. The higher activity of JEF compared with JME and other fractions as well as concentration of alkaloids and flavonoids in JEF suggest that these phytochemicals could be responsible for the significant antiplasmodial activity observed in this study. The confirmation of these observations, however, requires bioassay-guided isolation and quantification of the phytochemicals.

CONCLUSION

The study demonstrates the *in vitro* and *in vivo* antiplasmodial activities of *J. linifolia* based on its ethnomedicinal relevance. The extract and its ethyl acetate fraction elicit significant antiplasmodial and low cytotoxic activities in *in vitro* assay, which also correlates with the schizonticidal and curative effects during *Plasmodium berghei* infection in *in vivo* assay. The mid-polar fraction of *J. linifolia* represents a

promising source of lead antiplasmodial compounds.

DECLARATIONS

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Ethical approval

The protocol for the use of mice in this study was reviewed and approved by the Committee on Animal Ethics of the Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka (Reference No.: FPSRE/UNN/22/0002).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Concept and design- Nnadi Charles and Agbo Matthias; Data acquisition- Kuranen Fabian; data analysis/interpretation- Nnadi Charles and Kuranen Fabian; Drafting manuscript- Kuranen Fabian; critical revision of manuscript- Nnadi Charles and Agbo Matthias; statistical analysis- Kuranen Fabian; final approval- all authors.

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