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Evaluation of the *in vivo* antiplasmodial activity of ethanol leaf extract and fractions of *Jatropha gossypifolia* in *Plasmodium berghei* infected mice

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The study evaluates the *in vivo* antiplasmodial activity of ethanol leaf extract and fractions of *Jatropha gossypifolia* in *Plasmodium berghei* infected mice. Phytochemical, physicochemical analyses, median lethal dose (LD_{50}), effects on biochemical parameter were evaluated and the schizonticidal effects during early and established infections were investigated. The extract (50-200 mg/kg) was screened for antimalarial prophylactic and curative activities against 50 (20-30 g) *P. berghei* infected mice using standard method. The prophylactic and curative activity tests were repeated for the fractions of the extract. The phytochemical analysis done on *J. gossypifolia* leaves showed presence of tannins, saponins, phlobatannins, alkaloids, flavonoids, terpenoids and cardiac glycosides. It contained total ash (6.3%); acid insoluble ash value (3.8%), water soluble ash value (2.5%), ethanol extractive value (4.5%) and moisture content (7%). LD_{50} was 4472.14 mg/kg. There was significant increase in alanine transaminase (ALT), alanine phosphatase (ALP) and aspartate aminotransferase (AST) at 500 mg/kg on the 28th day, indicating liver injury at high dose and prolonged administration. The crude extract of *J. gossypifolia* (50-200 mg/kg) exhibited moderate prophylactic and significant ($P < 0.05$) curative activities in both day 4 and 7 tests with a mean survival time comparable to the standard drug, quinine 100 mg/kg. The fractions of the leaf extract of *J. gossypifolia* exhibited moderate to good prophylactic and curative activities, with ethyl acetate fraction eliciting the best activity in both test models. The leaf extract and fractions of *J. gossypifolia* administration are safe and possess good antiplasmodial activity, which confirmed its folkloric antimalarial medicinal use.

Key words: Malaria, antimalarial, *Plasmodium berghei, Jatropha gossypifolia*, toxicity, biochemical parameters.

INTRODUCTION

Malaria is a mosquito borne infectious disease of humans and other animals caused by parasitic protozoan belonging to the genus *Plasmodium*. The disease is transmitted by the biting of an infected female *Anopheles* mosquito, and the symptoms usually begin ten to fifteen days after being bitten. Malaria is typically diagnosed by

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the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests. Methods that use the polymerase chain reaction to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity (Caraballo, 2014; Nadjm and Behrens 2012).

Malaria can be associated with several serious complications among which include development of respiratory distress, encephalopathy, splenomegaly, hepatomegaly, hypoglycemia, and hemoglobinuria spontaneous bleeding and coagulopathy (Taylor et al., 2012; Adebayo and Krettli, 2011; Ferri, 2009; Korenromp et al., 2005; Beare et al., 2011).

Malaria in pregnant women is an important cause of stillbirths, infant mortality, abortion and low birth weight, particularly in *Plasmodium falciparum* infection, but also with *Plasmodium vivax* (Hartman et al., 2010). Malaria is widespread in the tropical and subtropical regions (Caraballo, 2014). Malaria is commonly associated with poverty and has a major negative effect on economic development (Gollin and Zimmermann, 2007). The World Health Organization reports that there were 198 million cases of malaria worldwide in 2013. This resulted in an estimated 584,000 to 855,000 deaths, the majority (90%) of which occurred in Africa (Murray et al., 2012; Filler et al., 2003).

Many antimalarial agents have been developed and used in the malaria treatment, with significant cases of drug failures and the attendant side effects associated with these agents. Majority of the rural dwellers depend on traditional medicine as a source of primary health care including malaria. *Jatropha gossypifolia* has many folkloric ethnomedicinal uses, including malaria treatment. It is called Bellyache bush, Cotton leaf and Physic nut. *J. gossypifolia* is native to tropical America, but is now cultivated widely in tropical countries throughout the world (Wikipedia, 2019; Csurhes, 1999).

Throughout tropical Africa, different parts of *J. gossypifolia* are used for a range of ethnomedicinal purposes. The oil-rich seeds and seed oil are used as a drastic purgative and emetic and to expel internal parasites. The leaves and bark have the same purgative effect. The oil is applied internally as abortifacient, and externally as rubefacient to treat rheumatic conditions and a variety of skin infections, although its use on the skin may also cause an irritative rash.

In Senegal the seed oil is also applied against leprosy and rabies. The sap has a widespread reputation for healing wounds, as a haemostatic and for curing skin problems. It is applied externally to treat infected wounds, ulcers, cuts, abrasions, ringworm, eczema, dermatomycosis, scabies and venereal diseases. The sap has a styptic effect and is used against pains and bee and wasp stings. Dried and pulverized root bark is made into poultices and is taken internally to expel worms and to treat oedema. A decoction of the leaves is taken to treat colic, stomach-ache and fever, including malaria (Abbiw, 1990; Csurhes, 1999).

Despite the grand variety of popular uses and the data from *Jatropha* species, *J. gossypifolia* has been scarcely studied regarding biological activities; with the exception of the antimalarial activity, some biological activities have been studied and listed as follows: antihypertensive action (Abreu et al., 2003); anti-inflammatory and analgesic action (Bhagat et al., 2011); wound healing action (Vale et al., 2006); haemostatic action; anti-cholinesterase action; antioxidant action (Kharat et al., 2011); contraceptive action; tocolytic action; antineoplasic action (Kupcan et al., 1970); neuropharmacological action (Apu et al., 2012).

Predicting the emergence and spread of resistance to current antimalarials and newly introduced compounds is necessary for planning malaria control and instituting strategies that might delay the emergence of resistance (Hasting et al., 2000). Throughout tropical Africa, different parts of are used for a range of medicinal purposes, but the antimalarial activity has been largely uninvestigated; making antimalarial evaluation of this potential biostore to become apt. The aim of this study is to evaluate the antimalarial activity of *J. gossypifolia* leaf extracts and fractions in mice infected with *Plasmodium berghei* using prophylactic and curative models, and also evaluate their toxicity profile.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *J. gossypifolia* were collected in April 2015 in Agulu, Anambra State, Nigeria. The plant was identified by a technologist in the Pharmacognosy and Traditional Medicine Department of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Nigeria.

Reagents and chemicals

Ethanol (JHD, China), n-hexane (JHD, China), ethyl acetate(JHD, China), water (NBC, Nig), ammonia solution (Shakti Chemicals, India), aluminium chloride (Neel Chemicals, India), chloroform, glacial acetic acid (JHD, China), ferric chloride (Ecosia Chemical, Hong Kong).

Equipment

Microscope (Finlab, Nig), syringes and needles (1, 2 and 10 ml capacity), electronic weighing balance (Ohaus Corp, USA), water bath (Serological, England), beaker (Pyrex; 10, 50, 100 and 1000 ml), spatula, measuring cylinder, refrigerator (Thermocool, England), cotton wool (Pyrex).
Organism: *Plasmodium berghei*

Preparation of the plant material

The leaves were put in water to remove dust and unwanted particle. They were spread on a clean flat tray and allowed to air dry at room temperature for two weeks. The dried leaves were pulverized with

an analytical milling machine. 605.72 g of the dried pulverized leaves was macerated in 2500 ml of ethanol for 72 h. The mixture was sieved using porcelain cloth and filtered with a filter paper. The filtrate was dried *in vacuo* at 40°C. The extract was stored in a refrigerator for use.

Preliminary phytochemical analysis

Qualitative phytochemical analysis of the crude powder of the leaves collected was determined according to standard procedure to identify the constituents present in the plant extracts (Sofowora, 1993; Trease and Evans, 1989).

Experimental animals and housing

Albino mice (158) of both sexes with an average weight in the range of 20-30 kg were selected for the experiment. They were obtained from the Zoology Department of University of Nigeria Nsukka, Enugu State. They were fed with growers mash, with free access to water. The mice were allowed to acclimatize for 7 days.

Collection and Identification of *P. berghei*

Three infected mice obtained from University of Nigeria Nsukka. Blood was collected from the tail of the infected donor mice and placed on a clean glass slide. The glass slide was placed horizontally on the working bench. The slide and spreader were held at a suitable angle (45°), the spreader was pulled back to touch the drop of blood on the slide and spread it along. The film was fixed with methanol and lowered into an already prepared Giemsa stain (1 ml of Giemsa +19 ml of buffer) and allowed to stain for 45 min. The slide was lifted off the bench and air dried. The parasitaemia was examined microscopically under oil immersion and parasitized cells were counted.

Acute toxicity study

The acute toxicity study of *J. gossypifolia* was carried out according to the method employed by Lorke (1983) method but modified, using a total of 17 mice (Bruce et al., 2016).

Anti-plasmodial activity of *J. gossypifolia*

In-vivo evaluation of the anti-plasmodial activity of *J. gossypifolia* was studied in two models using the method described by Ezenwa et al. (2017) and Adebayo et al. (2012):

(a) Prophylaxis test and
(b) Curative test

Prophylaxis test procedure

In this procedure, a total of 25 mice were used. They were grouped into 5 groups of 5 mice per group. The animals received treatment for 3 days as follows:

Group 1 received 5 ml/kg 5% tween 80 'per os', by mouth (P.O)
Group 2 received 22.5 mg/kg of sulfadoxine/pyrimethamine P.O
Group 3 received 50 mg/kg of extract
Group 4 received 100 mg/kg of extract
Group 5 received 200 mg/kg of extract

On the Day 4, all the animals were infected with *P. berghei* by a single 0.2 ml intraperitoneal administration of the diluted infected blood, which contains approximately 1×10^7 infected red blood cells. The animals were left for 72 h. Then a thin film blood smear was made from the tail vein stained in Giemsa stain and viewed under the microscope to examine the presence of parasitemia. Then the mean % parasitemia inhibition was calculated using the formula below:

Mean % Parasitemia inhibition = 100-(a/b x 100)

Where a = mean % parasitemia in treatment group, b = mean % parasitemia in control group

Curative test procedure

In this model a total of 25 mice were used. They were grouped into 5 groups of 5 mice per group. All the animals were infected with *P. berghei* by a single intraperitoneal administration of 0.2 ml of diluted infected blood, which contains approximately $1x10^7$ infected red blood cells. The animals were left for 72 h for the infection to be established. A thin blood film was made from the tail vein of each animal to confirm parasitemia and was termed the basal parasitemia. Then the animals received treatment as follows:

Group 1 received 5 ml/kg of 5% tween 80 P.O
Group 2 received 100 mg/kg of quinine P.O
Group 3 received 50 mg/kg of extract P.O
Group 4 received 100 mg/kg of extract P.O
Group 5 received 200 mg/kg of extract P.O

On Day 4 post treatment, the parasitemia was examined as above. Then the animals still continued treatment, with the parasitemia re-examined on day 7 post treatment. Then the mean % parasitemia inhibition was calculated using the relation.

Mean % parasitemia inhibition = 100-(a/b × 100)

Where a = mean % parasitemia on treatment day, b = mean % parasitemia on Day 0.

Prophylaxis test procedure for fractions

In this procedure, a total of 40 mice were used. They were grouped into 8 groups of 5 mice per group. The animals received treatment for 3 days as follows:

Group 1 received 250 mg/kg of N-hexane fraction of extract P.O
Group 2 received 500 mg/kg of N-hexane fraction of extract P.O
Group 3 received 250 mg/kg of Ethyl acetate fraction of extract P.O
Group 4 received 500 mg/kg of Ethyl acetate fraction of extract P.O
Group 5 received 250 mg/kg of aqueous fraction of extract P.O
Group 6 received 500 mg/kg of aqueous fraction of extract P.O
Group 7 received 22.5 mg/kg of sulfadoxine/pyrimethamine P.O
Group 8 received 5 ml/kg 5% tween 80 P.O

On the Day 4, all the animals were infected with *P. berghei* by a single 0.2 ml intraperitoneal administration of the diluted infected blood, which contains approximately 1×10^7 infected red blood cells. The animals were left for 72 h. Then a thin film blood smear was made from the tail vein stained in Giemsa stain and viewed under the microscope to examine for the presence of parasitemia. Then the mean % parasitemia inhibition was calculated using the relation.

Mean % Parasitemia inhibition = 100-(a/b × 100)

Where a = mean % parasitemia in treatment group, b = mean % parasitemia in control group.

Curative test procedure for fractions

In this model a total of 40 mice were used. They were grouped into 8 groups of 5 mice per group. All the animals were infected with *P. berghei* by single intraperitoneal administration of 0.2 ml of diluted infected blood such that it contains approximately 1×10^7 infected red blood cells. The animals were left for 72 h for the infection to be established. A thin blood film was made from the tail vein of each animal to confirm parasitemia and was termed the basal parasitemia. Then the animals received treatment as follows:

Group 1 received 250 mg/kg of N-hexane fraction of extract P.O
Group 2 received 500 mg/kg of N-hexane fraction of extract P.O
Group 3 received 250 mg/kg of Ethyl acetate fraction of extract P.O
Group 4 received 500 mg/kg of Ethyl acetate fraction of extract P.O
Group 5 received 250 mg/kg of aqueous fraction of extract P.O
Group 6 received 500 mg/kg of aqueous fraction of extract P.O
Group 7 received 100 mg/kg of quinine P.O
Group 8 received 5ml/kg 5% tween 80 P.O

On Day 4 post treatment, the parasitemia was examined as above. Then the animals still continued treatment the parasitemia was also examined on Day 7 post treatment. Then the mean % parasitemia inhibition was calculated using the relation.

Mean % parasitemia inhibition = 100-(a/b × 100) [Where a = mean % parasitemia on treatment day, b = mean % parasitemia on Day 0]

Physicochemical analysis

Physicochemical parameters on the plant materials were determined using the procedures described by Ezugwu et al. (2011).

Biochemical evaluation results

The effects of the administration of the leaf extract of *J. gossypifolia* on the biochemical parameters were evaluated using the method described by Ezenwa et al. (2017).

Statistical analysis

The data was analyzed using one-way analysis of variance (ANOVA). Data was tabulated as Mean ± SEM (Standard error of mean) $P < 0.05$ was considered significant.

RESULTS

Phytochemical analysis

The results of phytochemical analysis are presented on Table 1. Tannins, saponins, phlobatannins, flavonoids, alkaloids, terpenoids and cardiac glycosides are present.

Acute toxicity

The results of the median lethal dose test are presented on Table 2. The amount of the leaf extract of *J. gossypifolia* required to kill 50% of the test population LD_{50} is 4472.14 mg/kg.

Prophylaxis and curative tests

The results of the prophylaxis and curative tests of the extract are presented in Tables 3 and 4. Chemosuppression achieved in this study was dose dependent with chemosuppression rate of 85.42% at the highest dose 200 mg/kg when compared with the 91.70% of 22.5 mg/kg of sulfadoxine/ pyrimethamine (positive control) in Table 3. The curative effect of the 200 mg/kg crude extract which gives the percentage parasitaemia clearance of 88.23% (day 4) and 96.07% (Day 7) compared with the 100 mg/kg quinine (positive control) which also gives the percentage parasitaemia clearance of 88.37% (Day 4) and 93.02% (Day 7) in Table 4.

Prophylactic and curative tests results for the fractions

The results of the prophylaxis and curative tests of the fractions are presented in Tables 5 and 6. Chemosuppression achieved in this study was dose dependent with chemosuppression rate of 79.97% at the highest dose 500 mg/kg and 69.99% at the dose of 250 mg/kg, when compared with the 90.03% of 22.5 mg/kg of sulfadoxine/pyrimethamine (positive control) in Table 5. The curative effect of the 500 mg/kg ethyl acetate fraction which gives the percentage parasitaemia clearance of 66.69% (Day 4) and 87.15% (Day 7) and 250 mg/kg of ethyl acetate fraction which also gives the percentage parasitaemia clearance of 58.52% (Day 4) and 75.64% (Day 7), compared with the 100 mg/kg quinine (positive control) which gives the percentage parasitaemia clearance of 85.37% (Day 4) and 90.27% (Day 7) in Table 6.

Statistical comparison of results

The statistical comparisons of the prophylactic and curative test results for the crude extract are presented on Tables 7 and 8 respectively. In the prophylactic test result, the result of positive control compared those of 100 and 200 mg/kg extract treatments. There was no significant statistical difference in these treatments (Table 7). In the curative test result, the result of positive control compared those of 50, 100 and 200 mg/kg extract treatments. There was no significant statistical difference in these treatments at Days 4 and 7, except for the curative treatment group (Table 8).

Comparison of prophylaxis and curative test results for fractions

The comparison of the prophylactic test results of the fractions with the naïve group showed a significant difference in all the cases of comparison except for 250

Table 1. Phytochemical analysis.

Tests	Results
Tannin	+
Saponin	+
Phlobatannins	+
Flavonoids	+
Steroids	–
Alkaloids	+
Terpenoids	+
Cardiac glycosides	+

+ = Present-- =Absent.

Table 2. Acute toxicity result.

Phase	Dose (mg/kg)	No. of death	Inference
1	10	0/3	Active
	100	0/3	Active
	1000	0/3	Active
2	2000	0/2	Active
	3000	0/2	Active
	4000	0/2	Calm
	5000	½	Dead

$LD_{50} = \sqrt{axb}$ Where a = the highest dose that did not kill, b= the lowest does that killed

$\sqrt{4000x5000}$ $\sqrt{20000000}$ = 4472.14 mg/kg

LD_{50}= 4472.14 mg/kg .

Table 3. Prophylaxis test result of crude extract.

Group	Treatment	Mean parasitemia	Percentage prophylaxis
1	50 mg/kg extract	2.3 ± 0.2550	52.10
2	100 mg/kg extract	1.3 ± 0.3391	72.92
3	200 mg/kg extract	0.7 ± 0.1225	85.42
4	22.5 mg/kg of Sulfadoxine and Pyrimethamine	0.4 ± 0.1871	91.70
5	0.5 ml 5% Tween 80	4.8 ± 0.0.4062	0.00

mg/kg aqueous fraction of extract (Table 9). The comparison of the curative test results of the fractions with the basal showed a significant difference with the positive control, 250 and 500 mg/kg ethyl acetate fraction at Day 4; at Day 7 there was a significant difference with the positive control, 250 and 500 mg/kg ethyl acetate fraction, and moderately significant difference with 500 mg/kg n-hexane fraction (Table 10).

Physicochemical analysis

The result of the physicochemical analysis is presented on Table 11, showing a total ash value of 6.3% and a moisture content of 7.0%.

Biochemical evaluation

The result of the biochemical evaluations is presented on Tables 12 to 14. The result showed elevated effect of extract on AST at 500 mg/kg treatment at Days 14 and 28 in Table 12 and the elevated effect of extract on ALT and ALP at 125 mg/kg treatment at Days 14 and 28 in Tables 13 and 14.

DISCUSSION

Traditional medicinal plants have proved to be rich sources of new drugs coupled with the fact that antimalarial drugs in use presently were either obtained

Table 4. Curative test result of crude extract.

Group	Treatment	Basal Parasitemia	Day 4 Parasitemia	Day 4 % Curative	Day 7 Parasitemia	Day 7 % Curative
1	50 mg/kg extract	6.0 ± 0.65	1.5 ± 0.47	75.00	0.8 ± 0.25	86.67
2	100 mg/kg extract	0.7 ± 0.88	0.8 ± 0.20	85.96	0.5 ± 0.15	91.22
3	200 mg/kg extract	5.1 ± 0.57	0.6 ± 0.29	88.23	0.2 ± 0.12	96.07
4	100 mg/kg quinine	4.3 ± 0.86	0.5 ± 0.22	88.37	0.3 ± 0.12	93.02
5	0.5 ml 5 % Tween 80	4.4 ± 0.50	6.2 ± 0.77	-40	11.1 ± 2.15	-177.40

Table 5. Prophylactic test result for the fractions.

Group	Treatment	Mean parasitemia	% prophylaxis
1	250 mg/kg N-hexane fraction	8.33 ± 0.333	37.51
2	500 mg/kg N-hexane fraction	6.67 ± 1.202	49.96
3	250 mg/kg Ethyl acetate fraction	4.00 ± 0.574	69.99
4	500 mg/kg Ethyl acetate fraction	2.67 ± 0.667	79.97
5	250 mg/kg aqueous fraction	11.67 ± 0.333	12.45
6	500 mg/kg aqueous fraction	9.00 ± 1.528	32.48
7	22.5 mg/kg Sulfadoxine and Pyrimethamine	1.33 ± 0.333	90.03
8	0.5 ml 5% Tween 80	13.33	0.00

Table 6. Curative test result for the fractions.

Group	Treatment	Basal parasitemia	Day 4 parasitemia	Day 4 percentage curative	Day 7 parasitemia	Day 7 percentage curative
1	250 mg/kg N-hexane fraction	10.67 ± 0.882	8.33 ± 1.202	21.93	7.00 ± 1.000	34.39
2	500 mg/kg N-hexane fraction	11.00 ± 1.155	8.67 ± 0.882	21.18	6.00 ± 1.000	45.45
3	250 mg/kg Ethyl acetate fraction	13.67 ± 0.333	5.67 ± 0.667	58.52	3.33 ± 0.333	75.64
4	500 mg/kg Ethyl acetate fraction	13.00 ± 1.155	4.33 ± 0.882	66.69	1.67 ± 0.333	87.15
5	250 mg/kg aqueous fraction	11.00 ± 1.530	10.00 ± 1.530	9.09	8.00 ± 1.000	27.27
6	500 mg/kg aqueous fraction	13.67 ± 0.333	10.00 ± 1.528	28.85	7.68 ± 1.202	43.82
7	100 mg/kg Quinine	13.67 ± 0.333	2.00 ± 0.577	85.37	1.33 ± 0.333	90.27
8	0.5ml 5% Tween 80	12.67±1.667	16.00±0.577	-26.28	18.67 ±0.882	.47.36

directly from plants or developed using chemical structures of plant-derived compound as templates. The feasibility of discovering new potent antimalarials from traditional medicinal plants is very promising. Therefore, this study demonstrates the *in vivo* antiplasmodial activity of the ethanol leaf extract and fractions of *J. gossypifolia* in *P. berghei* infected mice.

The preliminary phytochemical analysis done on *J. gossypifolia* leaves showed that it contains different secondary metabolites such as tannin, saponin, phlobatannins, flavonoids, terpenoids and cardiac glycosides. This correlates with an earlier study done by Murugalakshmi et al. (2014) which reveals the presence of alkaloids, tannins, flavonoids, phenolic compounds, steroidal sapogenins (saponins), unsaturated sterols, triterpenoids and essential oils. The anti-plasmodial activity observed in many plants is assumed to result from single or combined action of the metabolites which could be the same for the present study. These constituents have been found in other natural plant products which possess antiplasmodial activity. The alkaloid constituent of *J. gossypifolia* explains its relevance in treatment of malaria (Vijayta et al., 2015).

The different classes of alkaloids were believed to block protein synthesis in *P. falciparum*. Triterpenoid and steroid saponins have been found to be detrimental to several infectious protozoans such as *P. falciparum*. Tannins are complex phenol polymers which have

Table 7. Statistical comparison of the prophylaxis results.

Compared variables for prophylaxis	Significance	P value
22.5 mg/kg of sulfadoxine/pyrimethamine vs. 50 mg/kg extract	**	P < 0.01
22.5 mg/kg of sulfadoxine/pyrimethamine vs. 100 mg/kg extract	NS	P > 0.05
22.5 mg/kg of sulfadoxine/pyrimethamine vs. 200 mg/kg extract	NS	P > 0.05
22.5 mg/kg of sulfadoxine/pyrimethamine vs. Tween 80	**	P < 0.01
50 mg/kg Crude Extract vs. 100 mg/kg Crude Extract	*	P > 0.05
100 mg/kg Crude Extract vs. 200 mg/kg Crude Extract	NS	P > 0.05

NS= not significant; *= moderately significant; **= significant.

Table 8. Statistical comparison of the curative results.

Compared variables for curative	Significance	P value
100 mg/kg quinine vs. 50 mg/kg Crude Extract Day 4	NS	P > 0.05
100 mg/kg quinine vs. 100 mg Crude Extract Day 4	NS	P > 0.05
100 mg/kg quinine vs. 200 mg/kg Crude Extract Day 4	NS	P > 0.05
100 mg/kg quinine vs. 0.5ml Tween80 Day 4	**	P < 0.01
100 m/kg Quinine vs. 50 mg/kg Crude Extract Day 7	NS	P > 0.05
100 mg/kg quinine vs. 100 mg/kg Crude Extract Day 7	NS	P > 0.05
100 mg/kg quinine vs. 200 mg/kg Crude Extract Day 7	NS	P > 0.05
100 mg/kg quinine vs. 0.5 ml Tween80 Day 7	**	P < 0.01
50 mg/kg Crude Extract vs. 100 mg/kg Crude Extract Day 4	NS	P > 0.05
50 mg/kg Crude Extract vs. 200 mg/kg Crude Extract Day 4	NS	P > 0.05
100 mg/kg Crude Extract vs. 200 mg/kg Crude Extract Day 4	NS	P > 0.05
50 mg/kg Crude Extract vs. 100 mg/kg/kg Crude Extract Day 7	NS	P > 0.05
50 mg/kg Crude Extract vs. 200 mg/kg Crude Extract Day 7	NS	P > 0.05
100 mg/kg Crude Extract vs. 200 mg/kg Extract Day 7	NS	P > 0.05
50 mg/kg Crude Extract Day 4 vs. 50 mg/kg Extract Day 7	NS	P > 0.05
100 mg/kg Crude Extract Day 4 vs. 100 mg/kg Extract Day 7	NS	P > 0.05
200 mg/kg Crude Extract Day 4 vs. 200 mg/kg Extract Day 7	NS	P > 0.05

NS= not significant; *= moderately significant; **= significant.

Table 9. Comparison of prophylaxis test results for fractions.

Compared variables for prophylaxis	Significance	P value
0.5 ml 5% Tween 80 vs. 250 mg/kg N-hexane fraction of extract	**	P < 0.01
0.5 ml 5% Tween 80 vs. 500 mg/kg N-hexane fraction of extract	**	P < 0.01
0.5 ml 5% Tween 80 vs. 250 mg/kg Ethyl acetate fraction of extract	**	P < 0.01
0.5 ml 5% Tween 80 vs. 500 mg/kg Ethyl acetate fraction of extract	**	P < 0.01
0.5 ml 5% Tween 80 vs. 250 mg/kg aqueous fraction of extract	NS	P > 0.05
0.5 ml 5% Tween 80 vs. 500 mg/kg aqueous fraction of extract	**	P < 0.01
0.5 ml 5% Tween 80 vs. 22.5 mg/kg Sulfadoxine and pyrimethamine	**	P < 0.01

NS= not significant; *=moderately significant; **= significant.

antioxidant activity which may also contribute to the antimalarial activity due to inhibition of haemo-polymerization. Specifically, the parasitaemia suppression effect of the extract may be attributed to the presence of alkaloids (Murugalakshmi et al., 2014).

The acute toxicity result showed that the extract has good safety margin with LD_{50} of 4472.14 mg/kg, slightly toxic at doses higher than the LD_{50}. *J. gossypifolia* leaf

Table 10. Comparison of curative test results for fractions.

Group	Treatment	Compared variables for curative	Significance	P-value	Compared variables for curative	Significance	P-value
1	250 mg/kg n-hexane fraction	Basal vs. Day 4	NS	P > 0.05	Basal vs. Day 7	NS	P > 0.05
2	500 mg/kg N-hexane fraction	Basal vs. Day 4	NS	P > 0.05	Basal vs. Day 7	*	P < 0.05
3	250 mg/kg Ethyl acetate fraction	Basal vs. Day 4	**	P < 0.01	Basal vs. Day 7	**	P < 0.01
4	500 mg/kg Ethyl acetate fraction	Basal vs. Day 4	**	P < 0.01	Basal vs. Day 7	**	P < 0.01
5	250 mg/kg aqueous fraction	Basal vs. Day 4	NS	P > 0.05	Basal vs. Day 7	NS	P > 0.05
6	500 mg/kg aqueous fraction	Basal vs. Day 4	NS	P > 0.05	Basal vs. Day 7	*	P < 0.05
7	100 mg/kg quinine	Basal vs. Day 4	**	P < 0.01	Basal vs. Day 7	**	P < 0.01
8	0.5 ml 5% Tween 80	Basal vs. Day 4	NS	P > 0.05	Basal vs. Day 7	*	P < 0.05

NS= not significant; *=moderately significant; **= significant.

extract showed good therapeutic index and is safe for use at lower doses. Four day chemo-suppresive test showed that the crude extract achieved good suppression of the *Plasmodium* parasite in mice infected with *P. berghei*. Chemosuppression achieved in this study was dose dependent with chemosuppression rate of 85.42% at the highest dose 200 mg/kg when compared with the 91.70% of 22.5 mg/kg of sulfadoxine/ pyrimethamine (positive control). The chemosuppression rate of 85.42% at the highest dose 200 mg/kg recorded is quite good, when compared with the percentage suppression achieved by the ethanolic extract of *Alstonia boonei* which reported its chemosuppression rate of 54.68% at the highest dose of 800 mg/kg (Idowu et al., 2015). This reveals that the extract has a considerable high antiplasmodial effect. This may be due to the synergistic effect of some active constituents of the plants. Significant reduction of parasitaemia ($P < 0.05$) was observed in all groups of mice treated with the *J. gossypifolia* leaf extract when compared to the negative control.

The curative test revealed that the extract has a considerable high antiplasmodial effect. The curative effect was also dose dependent, but with a very small change with increase in dosage. The curative effect of the 200 mg/kg crude extract which gives the percentage parasitaemia clearance of 88.23% (Day 4) and 96.07% (Day 7) compared with the 100 mg/kg quinine (positive control) which also gives the percentage parasitaemia clearance of 88.37% (Day 4) and 93.02% (Day 7). This may also be due to the synergistic effect of some active constituents of the plants. Significant reduction of parasitaemia ($P < 0.05$) was observed in all groups of mice treated with the *J. gossypifolia* leaf extract when compared to the negative control. This result compared with the curative effect reported for the combination of powdered seed of *Picralima nitida*, stem bark of *Alstonia boonei* and leaves of *Gongronema latifolium* extract at 400 mg/kg body weight with the percentage parasitaemia clearance of 83.84%; and was in the same range with that of *A. boonei* leaves extract used alone, which with the percentage parasitaemia clearance of 81.36% (Iyiola et al., 2011). The antimalarial activity of the extracts could be attributed to the presence of some phytochemicals like alkaloids, tannins, saponins, flavonoids and terpenes present in the study. Specifically, the parasitaemia curative effect of the extract may be attributed to the presence of alkaloids.

The prophylactic and curative test results for the fractions of the extract *J. gossypifolia* are presented on Tables 5 and 6 respectively. The four day chemosuppresive test showed that the ethyl acetate fraction achieved good suppression of the *Plasmodium* parasite in mice infected with *P. berghei*. Chemosuppression achieved in this study was dose dependent with chemo-suppression rate of 79.97% at the highest dose 500 mg/kg and 69.99% at the dose of 250 mg/kg, when compared with the 90.03% of22.5 mg/kg of sulfadoxine/pyrimethamine (positive control). However, the ethyl acetate fraction exhibited greater percentage inhibition compared with other fractions. The curative test also revealed that the ethyl acetate fraction has a considerable high antiplasmodial effect.

The curative effect was also dose dependent, but with a very small change with increase in dosage. The curative effect of the 500 mg/kg ethyl

Table 11. Physicochemical analysis result.

Parameter	Values (%)
Total ash	4.3
Acid insoluble ash	3.8
Water insoluble ash	2.5
Moisture content	7.0
Ethanol extractive value	4.5

Table 12. Effect of extract on aspartate transaminase (AST).

Group	Treatment dose	Mean absorbance		
		Basal	14 Days	28 Days
1	125 mg/kg	35.573 ± 0.2133	36.01 ± 0.2829	36.48 ± 0.3124
2	250 mg/kg	34.316 ± 0.5340	37.12 ± 1.064	41.19 ± 2.801
3	500 mg/kg	38.09 ± 0.5180	44.55 ± 0.988**	49.56 ± 1.017**
4	0.5 ml of 15 % Tween 80	35.573 ± 0.2133	35.86 ± 0.1976	36.73 ± 0.7622

Values are mean ± SEM; n = 5; **means level of significant difference.

Table 13. Effect of extract on alanine transaminase (ALT).

Group	Treatment dose	Mean absorbance		
		Basal	14 Days	28 Days
1	125 mg/kg	72.52 ± 0.240	72.84 ± 0.4288	72.90 ± 0.563
2	250 mg/kg	63.01 ± 5.136	64.71 ± 5.078	66.16 ± 5.278
3	500 mg/kg	57.13 ± 0.671	60.45 ± 0.445*	63.48 ± 0.743**
4	0.5 ml of 15% Tween 80	62.46 ± 5.175	62.91 ± 5.197	63.003 ± 5.162

Values are mean ± SEM; n = 5; * and ** mean levels of significant difference.

Table 14. Effect of extract on alanine phosphatase (ALP).

Group	Treatment dose	Mean absorbance		
		Basal	14 Days	28 Days
1	125 mg/kg	70.863 ± 5.511	73.16 ± 5.131	74.38± 5.034
2	250 mg/kg	61.98 ± 0.979	63.996 ± 0.127	64.71 ± 0.600*
3	500 mg/kg	54.31 ± 1.091	57.295 ± 0.472*	58.75 ± 0.315**
4	0.5 ml of 15 % Tween 80	61.32 ± 0.575	61.34 ± 0.623	61.62 ± 0.875

Values are mean ± SEM; n = 5; * and **mean level of significant difference.

acetate fraction which gave the percentage parasitaemia clearance of 66.69% (Day 4) and 87.15% (Day 7) and 250 mg/kg of ethyl acetate fraction which also gave the percentage parasitaemia clearance of 58.52% (Day 4) and 75.64% (Day 7), compared with the 100 mg/kg quinine (positive control) which gave the percentage parasitaemia clearance of 85.37% (Day 4) and 90.27% (Day 7). The antiplasmodial property of the plant extracts may be attributed to presence of some phytochemicals which might have conferred some protective / antioxidative effect against oxidative stress induced in the host parasitized red blood cells (RBCs) by the malaria parasite (Nethengwe et al., 2012). When the positive control was compared with the test, there was no significant difference $P > 0.05$ in the rate at which they suppressed the parasitic load. Therefore, when the

antiplasmodial activity of Day 4 and 7 were compared there was also no significant difference P > 0.05.

The result of the physicochemical analysis showed moisture content value (7%), total ash value (6.3%), acid insoluble ash value (3.8%), water soluble ash value (2.5%), and ethanol extractive value (4.5%). Low moisture content is desirable for preservation and long shelf life of the plant part. The moisture content of the *J. gossypifolia* leaves which is 7% which is not more than 10% (British Pharmacopoeia, 2011). Total ash value indicates the absence of contamination, substitution and adulteration of the plant part. The total ash value which is 4.3% is desirable for leaves, which is not more than 5% (British Pharmacopoeia, 2011). Water soluble ash value is useful in ascertaining the quality and purity of the crude drug is 2.5% is desirable for leaves, which is not less than 2.0% (British Pharmacopoeia, 2011). Lastly, the ethanol extractive value of *J. gossypifolia* leaves is 4.5% (British Pharmacopoeia, 2011).

The result of the biochemical parameter screening, when compared to the basal values presented on Tables 12, 13 and 14 showed a significant increase in alanine transaminase (ALT), alanine phosphatase (ALP) and aspartate aminotransferase (AST) levels with increase in the dose of the extract. There was significant difference in the value of the experimental animals when compared with the basal control, providing sufficient evidence that the impact was due to the extract. This has also been reported in earlier findings on other traditional medicinal plants such as *Chrozophora senegalenesis* (Ali et al., 2011) and ethanolic extract of *Magnifera indica* (Ogbe et al., 2012). There was significant difference in the value of the experimental animals when compared with the two controls, providing sufficient evidence that the impact was due to the extract. The level of the ALP at 400 mg/kg showed no difference in all the experimental animals, suggesting that the leakage in AST and ALT was from the liver and not bile duct. Considering the observation in the three parameters, it can be adduced that the extract had an impact on the liver, thereby allowing a leakage of AST and ALT into the blood stream (Idowu et al., 2015).

Conclusion

The results of this study have shown that the ethanol leaf extract and fractions of *J. gossypifolia* possess antiplasmodial property as seen in its ability to suppress malaria in *P. berghei* infection in the two models evaluated. The leaves also contains the normal range of values for moisture content values, ash values and extractive values as specified above, which is an important aspect of standardization so as to establish the correct identity of the crude drug before inclusion in the Pharmacopoeia. This justifies the traditional use of this plant as malaria remedy. It also provides scientific basis for the continuous use of this plant in the treatment of malaria in parts of Nigeria. This study could form basis for further research on this plant, which has exhibited antiplasmodial activity, for possible development of antimalarial drug or preparation.

Ethical statement

All authors hereby declare that the principles of laboratory animal care "(NIH publication No 85-23, revised 1985), were adopted. The research work did not involve human subjects, thus consent to participate is not applicable here.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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