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IN-VIVO ANTI-PLASMODIAL ACTIVITYOF METHANOLIC LEAF EXTRACTS OF *JATROPHA CURCAS* ON *PLASMODIUM BERGHEI* (NK 65) INFECTED MICE

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ABSTRACT

In vivo anti-plasmodial activity of methanol extracts and solvent fractions of the leaf extract of *Jatropha curcas* on *Plasmodium berghei* (NK-65) infected mice was investigated. The plant extract was prepared by cold maceration and crude methanol extract were partitioned using n-hexane, ethyl acetate and n-butanol. Phytochemical test were carried out to identify the secondary metabolites present in the plants extract. The antiplasmodial activity of the extracts and its fractions was evaluated using Peter four (4) days suppressive test. One-way analysis of variance (ANOVA) followed by least significant difference (LSD) *post-hoc* test was used to determine statistical significance. Qualitative phytochemical analysis showed the presence of saponin, flavonoid, alkaloids, tannin, triterpene, carbohydrates, steroids, glycosides and cardiac glycosides in methanol extract, ethyl acetate fraction and butanol fraction while hexane fraction shows the presence of steroids, glycoside and cardiac glycosides. At the end of the four (4) days suppressive test, the effect of the methanol extracts and its fractions of *Jatropha curcas* leaf on parasitaemia suppressive activity were dose-dependent for various extract treated group and crude methanol extract having the highest suppressive activity (67.15%) at 750mg/kg. These findings show *Jatropha curcas* leaf has antimalarial activity and the extracts is safe for oral use. The study recommends that further research on aqueous and methanol extract and fractions of *Jatropha curcas* leaf could be carried out to isolate, identify and characterize the active compound from this plant.

Keywords: Anti-plasmodial, *Jatropha curcas*, *Plasmodium berghei*, Mice

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Introduction

Malaria is a debilitating disease that is transmitted to humans by the bite of female *Anopheles* mosquito infected by *Plasmodium* species (1). The mosquito bite introduces infective stage (sporozoite) of *Plasmodium* from the mosquito's saliva into the human blood (2). The parasites travel to the liver where they mature and reproduce. Five species of *Plasmodium* (*P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*) can infect and be transmitted between humans with most fatal cases caused by *P. falciparum* (2). World Health Organization reported that, about 3.4 billion people live in areas at risk of malaria transmission in 106 countries and territories (2). It was also reported that malaria caused 198 million clinical episodes and 500,000 deaths annually around the world (3). Rollback malaria programme reported that malaria remained one of the major public problems in Sub-Sahara Africa (4). Moreover, the people at risk of this infectious disease are children under the age of five years and pregnant women (5). According to Ugwu *et al.* over 729 per 100,000 children of less than five years of age in Nigeria die annually due to malaria infection (6); the disease is prevalent in tropical and sub-tropical regions and is a major obstacle to economic advancement of the region's leading to poverty and malnutrition. In pregnant women, malaria often results in anemia, spontaneous abortion, preterm babies with low-birth-weight and neonatal deaths among others. Despite the substantial progress made in the treatment of parasitic diseases, malaria remains a significant therapeutic challenge especially because of the widespread resistance of malaria parasites to currently available anti-malarial agents, the resistance of the mosquito vectors to currently available insecticides, the limited success in the development of malarial vaccines and the debilitating adverse reactions of conventional anti-malarial drugs. These have stimulated the search for new pharmacologically active agents that can overcome these barriers (7). There is a long-standing tradition for the use of phytomedicines for the treatment of malaria. Plants of medical importance have an essential role in the development of new drugs and ensuring an efficient healthcare system of many nations including Nigeria. According to Newman *et al.* (8), at least 119 chemical substances originating from plants can be considered as important drugs for the treatment of various ailments across many nations. Secondary metabolites of plants (phytochemicals) with previously unknown pharmacological activities have been extensively investigated as medicinal agents (9). These secondary metabolites differ from plant to plant and include such examples as: anthraquinones, flavonoids, glycosides, saponins, tannins and etc. Plants also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for the treatment of different diseases (9).

Jatropha curcas are commonly known as physic plant or purging nut or big purginant and they are drought resistant large shrub, belonging to the family Euphorbiaceae. There are different species of *Jatropha* but *J. curcas* is one of commonest species of plants found in Nigeria. Traditionally *Jatropha curcas* is used for the treatment of fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism (10,11). Various scientific works have authenticated the numerous medicinal properties of *J. curcas*, Sarkiyayi *et al.* (12) reported the antiplasmodial and hepatoprotective effect of aqueous stem bark extract of *Jatropha curcas* on *Plasmodium beighei* infected mice. The ethanol leaf extract was reported by Ehsaan *et al.* (13) to possess antioxidant and anti-inflammatory effects, while the hepatoprotective effect of the methanol leaf extract on cadmium induced toxicity was demonstrated by Adejumobi *et al.* (14) using rabbits. Ehsaan *et al.* (13) reported the anti-cancer effect of the metanolic leaf extract of the plant. Studies also reported the presence of antibacterial agents in different parts of *J. curcas* (15). The aim of this study is to investigate the *in vivo* anti-plasmodial activity of methanolic leaf extract of *J. curcas* and its fractions on *Plasmodium berghei* infected mice.

Materials and Methods

Plant Collection: Leaves of *Jatropha curcas* were collected from the *Jatropha* plantation of National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State Nigeria and authenticated at the Herbarium of the Department of Botany, Ahmadu Bello University, Zaria with a voucher No. 01911.The plant was washed thoroughly under running tap water to remove dust and other unwanted particles. The leaves were air dried under shade at room temperature.

Extraction and Solvent Partitioning

Plant Extraction

The dried leaves was crushed to powder with pestle and mortar and Five hundred gram (500g) of the plants powder was cold macerated in 1000ml of 70% methanol. The methanol extracts was periodically shaken for 48hours and filter. The procedure was repeated three times to exhaustively extract the constituents of the plant materials. The filtrate was kept and two-third of the initial solvent was added to the content of funnel, shaken and allowed to stand for another 24hours, after then it was filtered. The filtrate was pooled together and then solvent was remove in vacuum using rotary evaporator to obtained crude methanol extract and kept for the investigation.

Solvent Partitioning

The crude methanol extract was then partitioned using solvents of different polarities. The solid crude methanol extract was dissolve in distilled water to form an aqueous extract which was then serially partitioned with N-hexane, Ethyl acetate and N-butanol in a separating funnel. Each partition process was repeated three times using equal volume of each solvent and similar fraction

were pooled together and concentrated in vacuum at 40ºC.

Phytochemical Screening

Phytochemical screening of the crude plant extract was carried out employing standard procedures and tests described by Trease and Evans, (16).

Study Animals

Seventy five albino mice were bought from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in cages under standard laboratory conditions and fed on standard pelleted diet (growers mash). They were acclimatized to the laboratory environment for 10 days with food and water provided *ad libitum*. Ethical clearance was sought for from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval No. ABUCAUC/2020/67.

Study Drugs

The standard drug (chloroquine) and extract was used in the antiplasmodial study and they are administered orally with the aid of an insulin syringe and needle.

Malaria Parasite

The malaria parasite (*Plasmodium berghei* NK-65) was obtained from a donor Mouse in National Institute for Medical Research (NIMR), Lagos, Nigeria. The parasite was maintained by sub-passaging into healthy mice and transported to the animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria in a standard cage.

Acute Toxicity Test

The LD_{50} of the crude methanolic extract and its fraction of the leaves was tested using modified Locke's, (17) method to determine safety. The crude methanolic leaves extract and its fraction of *J. curcas* were administered in doses of 1000, 100 and 10 mg/kg body weight to three groups of mice (n = 3) in the first phase. While the second phase concentration were 1600, 2900 and 5000 mg/kg body weight to three groups of mice (n = 3). The mice were kept under same conditions and observed for toxicity signs including change in behavior, loss of appetite and mortality for 24 hours.

Parasite Inoculation

The inoculation of the parasite was carried out by determining both the percentage parasitemia and erythrocytes count of the donor mouse using a haemocytometer and appropriate dilutions of the infected blood with isotonic saline. Each mouse in all the groups except the group 1 mice were inoculated intraperitoneally with 0.2 ml of infected blood containing about $1x10^7$ *Plasmodium berghei* parasitized red blood cells.

Animal Grouping

Seventy albino mice were divided into fifteen (14) groups of five (5) mouse each. Groups 1-14 were inoculated with the rodent malaria parasite *Plasmodium berghei* from the same donor mouse.

Groups 3-14 were treated with 250, 500 and 750 mg/Kg of the crude methanolic extract, n-hexane, n-butanol and Ethyl acetate fractions of *J. curcas* leaves respectively. Group 2 was treated with 20 mg/Kg body weight of Chloroquine (positive control) while 10 mL/kg of normal saline was administered to group 1 which served as a negative control.

Evaluation of Suppressive Activity of the Extract (Peter's 4-Day Test)

Peter's 4-day test was used to evaluate the activity of the extracts against early *P. berghei* infection in Mouse (18). The mice were inoculated by intraperitoneally with 1×10^7 infected erythrocytes. Treatment commenced three hours after mice had been inoculated with the parasite on day 0 and then continued daily for four days from day 0 to day 3 with 250, 500 and 750 mg/kg of the crude extract and its fraction orally. Group 2 (positive control) was also treated from day 0 to day 3 with 20 mg/kg of chloroquine. After treatment was completed, thin blood film was prepared from the tail of each animal on day 5 to determine parasitemia. The film was then stained with Giemsa stain to reveal parasitized erythocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the negative control as follows:

$$\%\ \text{Suppression} = \frac{\text{APC} - \text{APT}}{\text{APC}} \times 100$$

Where **APC** = Average Parasitaemia in the Negative Control

APT = Average Parasitaemia in the Treated group

Data Analysis

Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (least significant difference). Differences between means was considered significant at 5% level of significance ($P\leq0.05$) using SPSS (Statistical Package for Social Sciences) version 23.

Results and Discussion

Qualitative phytochemical constituents of crude methanol extract of *J. curcas* leaf and it fractions were presented in Table 1. The results reveal that anthraquinone was absent in all the extract while presence of saponin, flavonoid, alkaloids, tannin, triterpene, carbohydrates, steroids, glycosides and cardiac glycosides were detected in, methanol extract and butanol fraction. The table also show the presence of alkaloid, steroids, glycoside and cardiac glycosides in hexane fraction. While flavonoid, alkaloids, tannin, triterpene, carbohydrates, steroids, glycosides and cardiac glycosides are presence in Ethyl acetate fraction.

Table 1: Phytochemical screening of *Jatropha curcas* Leaf Extracts and Fractions

Phytochemicals Constituents	Methanol	Hexane	Ethyl Acetate	Butanol
Anthraquinone	-	-	-	-
Saponin	+	-	-	+
Flavonoids	+	-	+	+
Alkaloids	+	+	+	+
Tannin	+	-	+	+
Triterpene	+	-	+	+
Carbohydrates	+	-	+	+
Steroids	+	+	+	+
Glycosides	+	+	+	+
Cardiac Glycosides	+	+	+	+

Key: + Present - Absent

Table 2 revealed the median lethal dose of aqueous and methanol leaf extracts of *J. curcas* and its fractions. No death was recorded after the oral administration up to a dose of 5000mg/kg body weight for the extracts and its fractions. LD50 was calculated to be ≤5,000 mg/kg.

Table 2: Median Lethal Dose (LD_{50}) Determination of Crude Methanol Extract of *Jatropha curcas* Leaf and its Fractions

Phase	Dosage of leaf extract/fractions (mg/kg)	No. of Death/No of Mice Used	% Mortality
I	10	0/3	0.0
	100	0/3	0.0
	1000	0/3	0.0
II	1600	0/3	0.0

	2900	0/3	0.0
	5000	0/3	0.0

At the end of the four (4) days suppressive test, the extracts and its fraction showed a dose dependent and significant ($P<0.05$) decrease in parasitaemia level of which methanol extracts and butanol fraction at 750 mg/kg exhibited the highest significant percentage suppression (67.15 and 62.15%) compared to the other extract/fraction treated group while chloroquine (20 mg/kg) showed 90.87% suppression (Table 3).

Table 3: Peters 4-days Suppressive Effects of Methanol Leaf Extracts of *Jatropha curcas* and its Fractions against *Plasmodium berghei* Infected Mice

Treatment	Dosage (mg/kg)	Parasitaemia Level	% Suppression
Normal Saline	10 ml/kg	10.41 ± 0.45^{f}	-
Chloroquine	20	0.95 ± 0.12^{a}	90.87
Methanol Extract	250	4.30 ± 0.23^{c}	58.69
	500	4.09 ± 0.32^{bc}	60.71
	750	3.42 ± 0.34^{b}	67.14
Ethyl Acetate Fraction	250	4.67 ± 0.22^{bcd}	55.14
	500	4.39 ± 0.20^{bcd}	57.82
	750	4.02 ± 0.02^{de}	61.38
Hexane Fraction	250	5.01 ± 0.14^{bc}	51.87
	500	4.92 ± 0.44^{bc}	52.73
	750	4.28 ± 0.36^{bcd}	58.88
Butanol Fraction	250	4.75 ± 0.25^{bcd}	54.37
	500	4.20 ± 0.10^{bcd}	59.65
	750	3.94 ± 0.14^{de}	62.15

Key: Values are Mean±SD. Mean with different superscript across the row are significantly different (P≤0.0

Discussion

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (19). This plant based traditional medicinal system continues to play an essential role in health care, with about 80% of the world` inhabitants relying mainly on traditional medicines for their primary health care (20). The successful extraction of bioactive compounds from plants, according to Parekh *et al*. (21) is largely dependent on the type of solvent used in the extraction procedure. Effective extraction from the dried plant material was achieved using different solvent. The dried plant material was extracted using methanol, ethyl-acetate, butanol and n-hexane to obtain the bioactive compounds present in the plant under pharmalogical investigation. Willcox *et al.* (22) ascertain that organic solvent extractions are used as a good alternative in evaluating the antimalarial activities of plants, as organic solvents are able to extract a broad spectrum of chemical constituents. Even though, it is interesting to note that most medicinal plant preparations are conducted in aqueous solvents (water) traditionally, it is noteworthy to observe the effect of organic solvents on the plant material and to describe them. The preliminary qualitative phytochemical screening of the methanol, ethyl-acetate and butanol extracts of the *J. curcas* leaves revealed the presence of tannins, alkaloid, saponins, flavonoid, carbohydrates, terpenoids, glycosides, steroids and cardiac glycosides. While n-hexane fraction only showed the presence of alkaloids, glycosides, cardiac glycosides and steroids. Anthraquinone was absent in all the extracts tested. The present of this secondary metabolites is in agreement with work of Sarkiyayi *et al.* (12) in aqueous extraction of stem bark of *J. curcas*. Also Dharani *et al*. (23) explained that common antimalarial plants used to treat malaria in traditional medicine contain secondary metabolites, such as alkaloids, terpenoids, coumarins, flavonoids, chalcones, quinines and xanthones. Alkaloids, terpenoids and tannins detected in *J. curcas* leaves have been implicated for their antiplasmodial activity in previous study (12). Quinine, one of the most important and oldest antimalarial drugs, belongs to the class of alkaloids. The observation that no death caused by an oral dose of 5000 mg/kg body weight of the extracts and its various fractions could imply the safety of the plant to be used in the treatment of malaria as also suggested in Sarkiyayi *et al.* (12). The acute toxicity result of the present study suggested that the oral medial lethal dose (LD50) of the extract could be greater than 5000 mg/kg body weight as per OECD guideline No 425. The experimental determination of lack of acute toxicity at the extract dose of up to 5000 mg/kg body weight of mice maybe the reason why it is used traditionally in the treatment of malaria and fever. Analysis of test results indicated significant parasitaemia suppression by all the doses of methanol extracts of *J. curcas* and its fractions as compared to the negative control

after the 4-day suppression test. *In vivo* antiplasmodial activity can be classified as moderate, good and very good if an extract displayed a respective percent parasite suppression equal to or greater than 50% at doses of 500, 250 and 100 mg/kg body weight per day (24, 25). Based on this classification, the crude extracts and its fractions of *J. curcas* are considered to have exhibited good antiplasmodial activity, with dose dependent suppression against *P. berghei* infection in mice. The parasite suppression exhibited by these extracts is comparable to results of former studies conducted on aqueous stem extract of *J. curcas* (12). The presence of phytochemicals such flavonoids, terpenoids, phenols, alkaloids, glycosides, tannins, steroids, and saponins in the extract which have earlier been shown to have antiplasmodial effects (26, 27), could be responsible for the antimalarial effect observed in this study. Specifically, the presence of alkaloids in this plant is in accordance with the report of Sarkiyayi *et al*. (12), who described the main active ingredients in stem bark of *J. curcas* as alkaloids, glycosides, saponin and terpenoids.

Conclusion and Recommendation

This study confirms that methanol leaf extract of *J. curcas* and its fraction has antiplasmodial activities. The preliminary qualitative phytochemical screening of the methanol, ethyl-acetate and butanol extracts of the *J. curcas* leaves revealed the presence of tannins, alkaloid, saponins, flavonoid, carbohydrates, terpenoids, glycosides, steroids and cardiac glycosides and methanol leaf extracts of *Jatropha curcas* and its fractions had a better suppressive anti-plasmodial activities at 750mg/kg. The study recommends that further research on crude methanol extract and fractions of *J. curcas* leaf could be carried out in order to isolate, identify and characterize the active compound from this plant.

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