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Research Article
Phyto-Therapeutic Potential of Ethanol Leaf Extract and Fractions of *Jatropha curcas* on *Trypanosoma brucei* Infected Albino Rats

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Abstract

Background and Objective: Trypanosomiasis is one the prevalent diseases that affect both humans and livestock in the tropics. Its treatment is currently bedeviled by a plethora of challenges including the toxicity of trypanocidal drugs and the development of resistance by the parasites. These limitations have prompted the search for alternative active substances from natural origin. In view of this, the phytotherapeutic potential of ethanol leaf extract of *Jatropha curcas* was assessed for suppressive and therapeutic antitrypanosomal activity in infected albino rats. **Materials and Methods:** A total of 45 rats were used for the study. The ethanol crude leaf extract of *J. curcas* was subjected to screenings for suppressive and therapeutic activities against *T. brucei brucei* infected-albino rats using standard procedure. There were 5 groups (A-E) of 5 rats each. All the groups were inoculated with 0.1 mL of the inoculum containing about 106 trypanosomes. For suppressive activity, animals in groups A and B were treated with 400 and 800 mg kg^{-1} (b.wt.) of the crude extract, respectively 2 hrs after infection for 5 days. Phytochemical screening, therapeutic and suppressive activity and haematological parameters were determined in the dried leaves extract and fractions using standard methods. **Results:** The treatment with 400 mg kg^{-1} of the extract caused a significant ($p<0.05$) decrease in parasitaemia on day nine post-treatment similar to the group administered with a reference drug. Flavonoids, alkaloids, tannins, steroids, phenols, saponins and terpenoids were detected in the extract. Improvement in body weight, packed cell volume and haemoglobin were observed in all the treated groups compared to the untreated control. Among the solvent fractions of *J. curcas* extract, the n-hexane fraction exhibited the highest trypanocidal activity. Leaf extract of *J. curcas* reduced parasitaemia and improved haematological parameters in trypanosomiasis-induced albino rats. Therefore, the extract has antitrypanosomal potential. **Conclusion:** The crude ethanol leaf extract of *J. curcas* possesses antitrypanosomal activity exhibiting both suppressive and therapeutic activities at different concentrations.

Key words: Antitrypanosomal, *Trypanosoma brucei*, *Jatropha curcas*, albino rats, fractions, phytochemicals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Trypanosomiasis, a disease of both humans and livestock, affects health as well as agricultural production, thus impacts negatively on development, especially in rural areas. Its eradication will have a tremendous positive impact on all the millennium development goals[1]. This vector-borne disease causes serious health and economic problems around the globe, especially in Sub-Saharan Africa. It is caused by several species of trypanosomes which are single-celled parasites transmitted by the bite of *Glossina* spp. (tsetse fly). These parasites invade the blood of their host and cause several changes such as cellular and biochemical changes in the blood of the patients. Infection by *Trypanosoma brucei* leads to increased red blood cell destruction which results in anaemia[2], immunodepression, reduced level of systemic antioxidants[3], hyperplasia of lymph nodes, spleen and liver[4], weight loss, infertility, decreased milk yield and abortion in affected animals[5]. These outlined changes, coupled with the struggle by the host to deal with the parasite may be responsible for the symptoms of African trypanosomiasis. These symptoms are aggravated as the illness progresses and eventually, death may occur after several years of untreated infection[6].

Treatment of the disease is still faced with problems of increasing parasite resistance, toxicity, narrow spectrum of activities, route of administration, drug regimen (period of treatment), scarcity of drugs and high cost of available drugs[7]. The use of herbal concoctions and decoctions for the treatment of trypanosomiasis is still viable because some of them have demonstrated potent trypanocidal activity[5,8-11].

Jatropha curcas L., belonging to the Euphorbiaceae family, is native to America, it is widely distributed all over many countries in Africa where it is used for various medicinal purposes. It is used medicinally in the treatment of bacterial and fungal infections as well as other diseases such as ulcers. *Jatropha curcas* leaves have been reported to contain a variety of bio-compounds such as steroid sapogenins, alkaloids and terpenoids of varied types. Examples of alkaloids isolated from the leaves include 5- hydroxypyrrolidin-2-one, pyrimidine-2 and 4-dione[12]. An ethanol extract of the leaves and twigs of *J. curcas* has shown both *in vivo* and *in vitro* against a specific type of lymphocytic leukemia[13]. In Nigeria, the leaves of *J. curcas* are mainly used for the treatment of jaundice[12]. Therefore, this work was carried out to determine the phyto-therapeautic potential of *Jatropha curcas* on *Trypanosoma brucei* infected albino rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Research Laboratory of the Department of Biology, Federal University of Technology, Owerri Imo State between the months of July and October, 2021. Owerri is the capital of Imo State found on longitude 5°30' North and 7°010' East. It lies in the tropical rainforest region of South Eastern Nigeria.

Parasites (*Trypanosoma brucei*): *Trypanosoma brucei* parasites were obtained from the Department of Veterinary Medicine (Parasitology and Entomology), University of Nigeria Nsukka. The parasites were maintained by serial passaging in rats until needed.

Plant materials: The leaves of *Jatropha curcas* (local name "Bobochi") were collected from Agbani in Enugu State. The plant samples used in this study were identified and authenticated by a Plant Taxonomist while a Voucher specimen of the plant in the Herbarium for reference.

Extraction and concentration of plant crude extract: A known quantity of 1 kg of air-dried pulverized *Jatropha curcas* leaves was extracted with 80% ethanol in 20% water (8:2 v/v) using the cold maceration method for 4 days[7], fresh solvents were introduced every 48 hrs. The resulting filtrate was pulled together and concentrated *in vacuo* first using a rotary evaporator at 50°C and later freeze-dried at 40°C. Crude extract yields were stored in air-tight bottles at 4°C to avoid biological degradation.

The percentage yield was calculated as[7]:

$$\text{Yield (\%)} = \frac{\text{Amount of extract obtained}}{\text{Amount of initial sample}} \times 100$$

Animal procurement and handling: Male and female albino rats weighing 103-205 g procured from Zoological garden of the Department of Zoology and Environmental Biology, University of Nigerian Nsukka were used for this study. The animals were kept in plastic rat cages and allowed to acclimatize for 1 week before the commencement of the experiments. They were fed with finisher's mash (Vital feeds, Nig. Ltd.) and water *ad libitum*.

Clearance and approval for conducive experimental conditions and humane use and handling of laboratory animals were given by the ethical committee of the Department of Zoology and Environmental Biology, University of Nigeria Nsukka.

Phytochemical screening: Phytochemical evaluation of the plant sample was carried out on the crude extract using standard methods by Sofowora[14] and Bero *et al.*[15] to identify the constituents.

Experimental design

Suppressive and therapeutic activities of crude ethanol leaf extract: A total number of 45 rats were used for the study. The ethanol crude leaf extract of *J. curcas* was subjected to screenings for the suppressive and therapeutic activities against *T. brucei* infected-albino rats according to the method of Dutta *et al.*[16]. There were 5 groups (A-E) of 5 rats each. All the groups were inoculated with 0.1 mL of the inoculum containing about 10^6 trypanosomes. For suppressive activity, animals in groups A and B were treated with 400 and 800 mg kg^{-1} body weight (b.wt.) of the crude extract, respectively 2 hrs after infection for 5 days.

For therapeutic activity, treatment with the crude extract was administered at the same dosage as the suppressive study after 7 days of infection for 5 consecutive days on the establishment of parasitemia. *Diminazene aceturate* which was used as a reference drug (positive control) was administered at 3.5 mg kg^{-1} once to the animals in group C. Animals in group D were infected but not treated (negative control) while those in group E were not infected but treated with 5% tween 80 in physiological saline and served as the normal group. Parasitemia in the blood of infected animals was monitored daily and estimated using the rapid matching method[17].

Assessment of haematological indices of infected rats treated with crude ethanol leaf extract: Determination of the effect of the ethanol crude leaf extract of *J. curcas* on the haematological indices of treated rats was evaluated. At the end of the treatment, blood samples were collected from the animals using the ocular puncture method for the analysis of Packed Cell Volume (PCV), Haemoglobin (Hb) concentration and total leucocytes count (TLC) according to Schalm *et al.*[18].

Fractionation of crude ethanol leaf extract: The crude ethanol leaf extract of *J. curcas* (27 g) was fractionated using the solvent-solvent extraction method with n-hexane, ethyl acetate, saturated n-butanol and water in the order of increasing polarity according to Wu *et al.*[19]. The extract was dissolved in 200 mL of the extracting solvents (ethanol and H_2O) in the ratio of 9:1 and was shaken with n-hexane (3×100 mL). The mixture was left to dry on the bench to yield n-hexane fraction. Methanol was evaporated from the remaining extract and diluted with distilled water to 200 mL and further fractionated by successive solvent extraction with ethyl-acetate (2×100 mL) and n-butanol saturated with water (3×100 mL). Each fraction was left to evaporate to dryness on the bench to yield n-hexane fraction (5.21 g), ethyl acetate fraction (10 g), n-butanol fraction (7.8 g) and water fraction (3.1 g), respectively[19].

***In vivo* assessment of the fractions for antitrypanosomal activity:** The n-hexane, ethyl acetate, n-butanol and H_2O fractions were evaluated for antitrypanosomal activity. Seven groups (A-G) of five rats each were inoculated with 10^6 trypanosomes per mL of blood using phosphate buffer saline. Two groups (A and B) served as positive (diminazene aceturate) and negative (infected and not treated) controls, respectively. Four groups (C-F) served as test groups while the last group (G) served as the normal group (not infected but treated with 5% tween 80 in psychological saline). The n-hexane was dissolved in 5% tween 80, while butanol, ethyl acetate and water fractions were dissolved in 5% carboxyl-methyl cellulose. On the establishment of parasitemia after 5 days post infection (pi), the reference drug and the fractions were administered orally to the infected rats at doses of 3.5 mg kg^{-1} (once) and 100 mg kg^{-1} for 5 days, respectively. The level of parasitemia was monitored for 28 days. The weight and survival period of experimental animals for each group were recorded.

Statistical analysis: All the data generated were expressed as Mean±Standard Deviation (SD) and analyzed statistically by Analysis of Variance (ANOVA). Statistical Package for Social Sciences IBM computer software package version 20 was used for the analysis and values of $p<0.05$ were considered as statistically significant.

RESULTS

Extract composition and texture: The percentage crude extract yield of *J. curcas* was 37%. The extract from *J. curcas* was dark-brown in colour, a slurry substance that was insoluble in water.

Phytochemical composition: The result of the quantitative and qualitative phytochemical screening of crude ethanol leaf extract of *J. curcas* was presented in Table 1. Flavonoids were in high concentrations in the extract. Alkaloids, terpenoids, saponins and tannins were found in moderate concentrations, while steroids and phenols were in small concentrations.

Table 1: Phytochemical composition of crude ethanol leaf extract of *J. curcas*

Phytochemical	Quantitative (mg/100 g)	Qualitative
Alkaloids	520.83±65.05	++
Flavonoids	1152.78±24.05	+++
Tannins	37.56±2.36	++
Reducing sugar	-	ND
Steroid	3.95±0.00	+
Carbohydrate	3012.08±132.23	++
Saponin (%)	41.66±2.88	++
Phenols (GAE)	2.02±0.62	+
Cardiac glycosides	-	ND
Terpenoids	116.13±14.38	++

Results were expressed as Mean±Standard Deviation, +: Present in small amount, ++: Moderately present, +++: Present in very high amount and ND: Not detected

The suppressive activity of the crude ethanol leaf extract of *J. curcas* was assessed at 400 mg kg^{-1} and 800 mg kg^{-1} body weight, respectively (Table 2). The extract was administered 2 hrs after infection to assess its ability to suppress parasite proliferation in the animal model. At 400 mg kg^{-1} dose, the infected treated rats showed maximum average parasitemia of 133.00±2.53 on the 5th day post-infection (pi) and minimum average parasitemia of 12.20±2.46 on the 2nd day pi. This dose was unable to clear parasites from the bloodstream of the rats rather the life span of the rats was prolonged till day 5 pi as against the infected untreated control group that died on day 3 pi. The same applies to the group treated with 800 mg kg^{-1}. At 800 mg kg^{-1} dose, the infected treated rats showed maximum average parasitemia per mL of blood of 123.20±1.38 on day 5 pi and the minimum average parasitemia of 4.60±0.68 was recorded on day 2 pi. Treatment with the reference drug (diminazene aceturate) caused complete suppression of parasitic proliferation within the group which resulted in the survival of all the animals in the group throughout the monitoring.

The result of the therapeutic activity of crude ethanol leaf extract of *J. curcas* was shown in Table 3. The experiment was carried out at 400 and 800 mg kg^{-1} b.wt., of the extract and 3.5 mg kg^{-1} of the reference drug. At 400 mg kg^{-1} b.wt., the rats showed a maximum average parasitemia of 66.67±2.73 on day 1 pi and a minimum average parasitemia of 21.00±0.00 on day 8 (pi). At 800 mg kg^{-1} body weight, the rats showed a maximum average parasitemia of 57.20±6.45 on day 1 pi and a minimum average parasitemia of 17.00±0.00 on day 8. On the 7th day pi, the untreated control group had maximum average parasitemia of 28.00±2.21 on day 1 pi and a minimum average parasitemia of 24.33±33 on day 2 pi, all the animals died on day 3 pi. The reference drug had 93.00±0.00 for the maximum on day 6 pi and 60.00±0.00 for the minimum average parasitemia on day 4 pi. The result showed a significant ($p<0.05$) decrease in parasitemia in the 400 mg kg^{-1} treated group and total clearance of parasite from the bloodstream of the animals on day 9 pi resulting in the survival of the animals throughout the monitoring period. The administration of the extract at 800 mg kg^{-1} dose showed slight therapeutic activity although parasites were not cleared from the blood but the life span of the animals was extended to the 8th-day pi compared to the untreated control group that died on the 3rd day pi.

Haematological changes in rats: Results of the haematological changes in rats treated with ethanol leaf extract of *J. curcas* were summarized in Table 4. The result revealed that treatment with *J. curcas* resulted in a significant ($p<0.05$) increase in Packed Cell Volume (PCV) and Haemoglobin (Hb) level compared to infected untreated control. The mean PCV values of the groups treated with 400 and 800 mg kg^{-1} were significantly ($p<0.05$) higher compared to the untreated control group (34.25±1.89). There was a non-significant ($p>0.05$) difference in the total leucocytes count (TLC) of the group of rats treated with 400 and 800 mg kg^{-1} b.wt., of the extract compared to the untreated control group.

Anti trypanosomal assessment of fractions of ethanol leaf extract of *J. curcas*: The result of the anti trypanosomal assessment of fractions of ethanol leaf extract of *J. curcas* was presented in Table 5. The result showed that the n-hexane fraction had the highest antitrypanosomal activity among the fractions. The maximum level of parasitemia per mL of blood (8.70±0.00) was recorded on day 4 pi and the minimum (1.40±0.20) on day 10 pi. Total clearance of parasites among this group was observed on the 11th day pi. The group of rats treated with water fraction had a maximum (9.00±0.00) and minimum (3.60±0.20) level of parasitemia on days 8 and 10 pi, respectively while the rats treated with n-butanol fraction exhibited the characteristic cyclic pattern of parasitemia peculiar to trypanosomes between day 5 and 7 pi. More so, a significant ($p<0.05$) reduction in the level of parasitemia was recorded from day 8 till day 13 without total clearance of parasites from the blood of the rats in the group compared to the infected untreated control. Total mortality of the rats in the infected untreated (negative control) group occurred on the 8th day pi.

Effect of different fractions of *J. curcas* on the weight of *T. brucei brucei* infected rats: The effect of different fractions of *J. curcas* on the weight of infected treated rats was presented in Table 5. The result showed that treatment with

Table 2: Suppressive activity of ethanol leaf extract of *J. curcas* on rats infected with *T. brucei*

	Days of experiment					
Experimental groups	1	2	3	4	5	6-28
Infected untreated	62.40±2.54^{d1}	69.00±1.90^{d1}	137.40±2.69^{d2}	Dead	Dead	Dead
Diminazene aceturate (3.5 mg kg^{-1})	0.00±0.00^{a1}	0.00±0.00^{a1}	0.00±0.00^{a1}	0.00±0.00^{a1}	0.00±0.00^{a1}	0.00±0.00^{1}
J. curcas extract (800 mg kg^{-1})	10.40±3.46^{b1}	4.60±0.68^{b1}	111.20±4.61^{b3}	92.00±5.59^{b2}	123.00±1.38^{b4}	Dead
J. curcas extract (400 mg kg^{-1})	14.20±2.96^{c1}	12.20±2.46^{c1}	127.20±2.24^{c2}	125.40±3.96^{c2}	133.00±2.53^{c2}	Dead

Mean values with different alphabets as superscripts in a column differ significantly (p<0.05)

Table 3: Therapeutic activity of crude ethanol leaf extract of *J. curcas* on rats infected with *T. brucei*

	Experimental groups				
Pi days	400 mg kg^{-1}	800 mg kg^{-1}	Infected untreated	Diminazene aceturate	Normal control
0	63.67±7.51^{d3}	67.20±4.21^{d3}	82.00±7.23^{a4}	76.67±2.33^{c2}	0.00±0.00^{a1}
1	66.67±2.73^{d3}	57.20±6.45^{d2}	81.67±8.29^{a3}	81.33±5.78^{c2}	0.00±0.00^{a1}
2	60.33±10.17^{d3}	49.60±9.15^{c2}	80.33±11.29^{a2}	70.33±5.36^{b12}	0.00±0.00^{a1}
3	52.00±6.81^{c23}	27.00±2.21^{b1}	Dead	70.00±0.58^{b12}	0.00±0.00^{a1}
4	44.00±8.50^{c2}	26.60±2.73^{b1}	Dead	60.00±0.00^{b1}	0.00±0.00^{a1}
5	43.67±8.67^{c2}	23.60±2.56^{b1}	Dead	81.00±0.00^{d2}	0.00±0.00^{a1}
6	28.67±3.38^{b1}	25.60±4.21^{b1}	Dead	93.00±0.00^{d3}	0.00±0.00^{a1}
7	22.33±2.03^{b1}	28.00±2.21^{b1}	Dead	90.00±0.00^{d3}	0.00±0.00^{a1}
8	21.00±0.00^{b1}	17.00±0.00^{a1}	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
9	00.00±0.00^{a1}	Dead	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
10	0.00±0.00^{a1}	Dead	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
11	0.00±0.00^{a1}	Dead	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
12	0.00±0.00^{a1}	Dead	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
13-28	0.00±0.00^{a1}	Dead	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}

Mean values with different alphabets as superscripts in a column differ significantly (p<0.05) and mean values with different numeric superscripts in a row differ significantly (p<0.05)

Table 4: Effect of *Jatropha curcas* crude leaf extract on hematological indices of rats infected with *T. brucei*

	Haematological parameters		
Experimental groups	PCV	Hb	TLC
Infected untreated	34.25±1.89^{c}	11.38±0.64^{c}	7075.00±1473.87^{a}
Diminazene aceturate (3.5 mg kg^{-1})	41.20±2.05^{a}	13.70±50.00^{a}	8050.11±2113.42^{a}
J. curcas extract (400 mg kg^{-1})	38.75±1.18^{b}	12.90±0.40^{b}	7900.00±651.92^{a}
J. curcas extract (800 mg kg^{-1}	38.00±2.89^{b}	12.67±0.95^{b}	7533.33±1277.15^{a}

Mean values with different alphabets as superscripts in a column differ significantly (p<0.05)

Table 5: *In vivo* therapeutic activity of fractions of *J. curcas* on albino rats infected with *T. brucei*

Pi days	n-butanol	Ethyl acetate	Water	n-hexane	Infected untreated	Diminazene aceturate	Normal group
1	6.00± 0.51^{b3}	7.50±0.21^{ab2}	7.92±0.07^{ab1}	7.32±0.15^{dc2}	5.70±0.43^{c4}	7.50±0.16^{ab2}	0.00±.00^{a1}
2	6.66±0.66^{ba2}	8.22±0.20^{aa1}	7.92±0.15^{ab1}	8.04±0.06^{aa1}	6.78±0.70^{a2}	0.00±0.00^{a1}	0.00±0.00^{a1}
3	6.66±0.73^{ba2}	8.40±0.25^{ab}	8.22±0.16^{aa1}	8.28±0.16^{aa1}	6.78±0.80^{a2}	0.00±0.00^{a1}	0.00±0.00^{a1}
4	6.66±0.67^{ba2}	8.58±0.07^{c1}	8.58±0.07^{c1}	8.70±0.00^{d1}	7.02±0.76^{d2}	0.00±0.00^{a1}	0.00±0.00^{a1}
5	6.66±0.67^{ba2}	8.82±0.12^{d1}	8.58±0.12^{c1}	5.38±0.16^{cb2}	7.08±.77^{d2}	0.00±0.00^{a1}	0.00±0.00^{a1}
6	7.02±0.76^{d2}	8.64±0.24^{d1}	8.40±0.21^{ab}	5.24±0.09^{b1}	7.20±0.80^{dd2}	0.00±0.00^{a1}	0.00±0.00^{a1}
7	7.02±0.76^{d2}	8.42±0.12^{ab}	8.54±0.14^{c1}	5.22±0.11^{b1}	7.20±0.80^{dd2}	0.00±0.00^{a1}	0.00±0.00^{a1}
8	3.30±0.03^{ca1}	5.22±0.13^{b1}	9.00±0.0^{d2}	5.22±0.13^{b1}	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
9	3.36±0.07^{ca1}	5.22±0.14^{b1}	3.60±0.20^{cd2}	5.14±0.18^{b2}	dead	0.00±0.00^{a1}	0.00±0.00^{a1}
10	3.42±0.10^{c2}	5.40±0.20^{bc4}	3.60±0.20^{cd2}	1.40±0.20^{ac1}	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
11	3.48±0.13^{c2}	5.40±0.20^{bc4}	Dead	0.00±0.00^{a1}	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
12	1.74±0.07^{b4}	3.60±0.20^{cd2}	Dead	0.00±0.00^{a1}	Dead	00.00±0.00^{a1}	0.00±0.00^{a1}
13	Dead	1.80±0.08^{b4}	Dead	0.00±0.00^{a1}	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
14	Dead	Dead	Dead	0.00±0.00^{a1}	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
15	Dead	Dead	Dead	0.00±0.00^{a1}	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}
16-28	Dead	Dead	Dead	0.00±0.00^{a1}	Dead	0.00±0.00^{a1}	0.00±0.00^{a1}

Mean values with different alphabets as superscripts in a column differ significantly (p<0.05) and mean values with different numeric superscripts in a row differ significantly p<0.05

n-butanol fraction of *J. curcas* did not improve the weight of the rats from the 1st week to the 2nd week. Treatment with ethyl acetate fraction increased the weight of *T. brucei brucei* infected rats significantly ($p<0.05$) from the 1st week of treatment to the 2nd week and was also significantly ($p<0.05$) higher compared to the group treated with n-butanol fraction. The weight of rats treated with n-hexane fraction was significantly ($p<0.05$) increased from the 1st week of treatment till the end of the monitoring period, this increment was much better than the effect of water and ethyl acetate fractions.

DISCUSSION

Preliminary screening of the plant sample used in this study showed the presence of Flavonoids, alkaloids, tannins, steroids, phenols, saponins and terpenoids. There was a progressive Improvement in body weight, packed cell volume and haemoglobin in all the treated groups compared to the untreated control. The initial screening of *J. curcas* crude extract for suppressive activity further resulted to mild suppressive activity at all concentrations administered. Oskoueian *et al.*[20] and James *et al.*[21] showed that the methanol leaf extracts of *J. curcas* exhibited antimalarial and cytotoxic activities. The antimicrobial potential of the aqueous leaf extracts of *J. curcas* has also been reported by Namuli *et al.*[22]. Aqueous leaf extract of *J. curcas* tested for anti-helminthic activity on Indian earthworm *Pheretima posthuma* indicated significant activity[23].

The qualitative and quantitative phytochemical screening revealed that *J. curcas* leaf extract has alkaloids in high concentration. This was in agreement with previous findings that reported the presence of alkaloids in *J. curcas* leaf[24-26]. Flavonoids are secondary metabolites that are mostly found in the polyphenolic compounds of plants[27]. It has been found that these compounds disrupt the parasite's cell membrane by complexing with its extracellular and soluble proteins and cell walls[28]. Alkaloids are known to possess strong pharmacological activity, especially on the central nervous system and the heart and as antiprotozoan agents. Ogbunugafor *et al.*[29] reported that alkaloids are the major phytochemical constituent of *Mitragyna ciliata* ethanol root extract. Many alkaloids especially isoquinoline and quinoline from Cinchona bark (Rubiaceae) have been reported to possess antitrypanosomal activity[30]. Common examples of alkaloids used as stimulants include caffeine, nicotine, morphine and the antimalarial drug, quinine[27].

Many secondary plant metabolites with antitrypanocidal properties have been identified[25,31]. The exact mechanism of the reported trypanocidal activity of *J. curcas* may probably be related to the activity of the bioactive components of the phytochemicals on the cellular mechanism of the parasite.

The increased PCV and Hb count recorded in the present study in the albino rats administered with ethanol leaf extract of *J. curcas* portrays the anti-anaemic potential of the extract in disease conditions such as trypanosomiasis. This was in consonance with Ngulde *et al.*[32], who found out an increase in PCV, RBC and hemoglobin (Hb) in albino rats treated with methanol root bark extract of *Cassia arereh*. Previous work on *Tridax procumbens* by Staubmann *et al.*[11] also recorded an increase in PCV and Hb count in rats treated with the ethyl acetate crude extract. The measurement of haematological indices, particularly PCV and Hb, has always been used as a simple screening test for anemia. Anemia is a condition in which the oxygen-carrying capacity of blood is reduced as a result of a reduction in the total number of red blood cells and also a reduction in PCV due to the reduced RBC[33]. Anaemia is not a specific disease, it is always a sign of an underlying disease. Increased haemolysis, diminished erythropoiesis and blood loss are factors that may cause anaemia.

The result showed that out of the four fractions obtained from the crude extract, n-hexane fraction showed the highest trypanocidal activity. This implies that the plant's antitrypanocidal activity lies more with the non-polar constituents. The reduction/clearance of parasitemia and prolonging of the lifespan of infected rats by n-hexane fraction could be attributed to the trypanosuppression potential of the bioactive constituents of the fraction[34]. These bioactive compounds normally act against some important life-sustaining processes such electron transport chain and other processes that protect against oxidative stress, thus eliminating the trypanosoma parasites[35]. Bioactive compounds especially alkaloids, terpenoids, phenolics and flavonoids have shown trypanocidal activity, alkaloids in particular have inhibited the growth of trypanosomes by intercalating with the DNA of trypanosomes[36] and inhibiting protein synthesis[37]. Moreover, flavonoids have demonstrated promising antitrypanosomal activities on the trypomastigote forms found in the bloodstream of mammals[38]. Further analysis of the effect of the fractions on the weight of infected rats indicated a significant increase in the weights of the treated rats as compared to their initial weight (baseline data). Thus, apart from the group treated with the reference drug, the group that recorded the highest weight increment was the same group that showed the best trypanocidal activity.

This shows that clearance of parasites from blood circulation improved the rate of feeding, absorption of nutrients and general fitness of the animals. These factors led to the prolonged survival period of the rats till the end of the monitored period. Reports have recommended that body weight be measured because it is a very sensitive index to the health status of an animal, rapid weight loss of an animal could either be a result of decreased feed or water consumption, which mostly leads to ill health or death[39,40].

This study has thus revealed the potential of *Jatropha curcas* in the treatment of *Trypanosoma brucei*. This is not farfetched as it contains many bioactive chemicals (phytochemicals) which has contributed to these overwhelming phyto-therapeutic potentials.

CONCLUSION

The crude ethanol leaf extract of *J. curcas* possesses antitrypanosomal activity exhibiting both suppressive and therapeutic activities at different concentrations. Similarly, the n-hexane fraction of the crude extract was identified as the most active fraction of *J. curcas*. Treatment of *T. brucei brucei* infected rats with the active fraction resulted in a reduction in parasitemia and prolonged the lives of the experimental animals compared to the untreated control. Therefore, the isolation of the active compound from this most active fraction will contribute immensely to the development of an active drug for the management and treatment of trypanosomiasis.

SIGNIFICANCE STATEMENT

This study was carried out to determine the phyto-therapeautic potentials of *Jatropha curcas* on *Trypanosoma brucei* infected albino rats. The current breakthrough recorded in the field of medicine, as a result of rapid use of both natural and synthetic antibiotics, has really alleviated the health of humans and decreased rate of mortality across the globe at large. The result from this study has add value to the field of phyto-medicine as an ethnopharmacological data base for the treatment of *Trypanosoma brucei* from plant source, it will help trado-medical practitioners in their application of herbal remedies as well as encourage the use of herbs by the locals. Chemically this will go a long way in reducing the use of synthesized drugs which are not only costly but often scarce.

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