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In Vitro Antitrypanosomal Activity and Phytochemical Screening of *Jatropha curcas* Aqueous Leaf Extract Against *Trypanosoma evansi*

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Abstract

African trypanosomiasis remains a major health and economic burden in sub-Saharan Africa, affecting both humans and livestock, while existing treatments are constrained by toxicity and increasing drug resistance. This study investigated the in vitro antitrypanosomal potential of an aqueous leaf extract of *Jatropha curcas* against *Trypanosoma evansi*, alongside its phytochemical composition. The extract was prepared through maceration and analysed qualitatively, revealing the presence of alkaloids, saponins, tannins, and cardiac glycosides, while other compounds such as flavonoids, steroids and terpenoids were absent. The antitrypanosomal activity was evaluated by exposing infected blood samples to varying extract concentrations (2.5–20 mg/mL) and monitoring parasite motility over 60 minutes. Results showed a clear concentration-dependent effect, with higher concentrations acting more rapidly. At 20 mg/mL, parasite movement stopped within 30 minutes, while lower concentrations required longer exposure times. The standard drug achieved faster immobilization, whereas no effect was observed in the untreated control. Generally, the extract confirmed mild but significant antitrypanosomal activity, likely linked to its bioactive constituents, particularly alkaloids. These findings suggest potential for further investigation, especially in isolating active compounds and validating their effectiveness in vivo.

Keywords: *Jatropha curcas*, *Trypanosoma evansi*, Antitrypanosomal, Phytochemical, *In vitro*, Aqueous extract.

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1. Introduction

Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) are debilitating diseases caused by haemoparasitic protozoans of the genus *Trypanosoma*, transmitted primarily by tsetse flies (*Glossina spp.*) (Wamwiri and Changasi; 2016; Maichomo *et al.*, 2021). These diseases pose a major threat to public health and livestock production across sub-Saharan Africa, negatively impacting the economy of endemic regions (Mboera *et al.*, 2014; Negesso *et al.*, 2016; Mwacalimba *et al.*, 2025). Key pathogenic species affecting livestock include *Trypanosoma congolense*, *T. vivax*, *T. brucei*, and *T. evansi*, while the subspecies *T. b. rhodesiense* and *T. b. gambiense* are responsible for sleeping sickness in humans (Aregawi *et al.*, 2019; Boundenga *et al.*, 2022; Kargbo *et al.*, 2022).

The management and treatment of trypanosomiasis are fraught with challenges. Currently available chemotherapeutic drugs are often old, expensive, and associated with severe side effects (Babokhov *et al.*, 2013; Altamura *et al.*, 2022; Barrett, 2025). Furthermore, the growing resistance of trypanosomes to these drugs, particularly in areas of high drug use, has reached alarming levels, underscoring an urgent need for new, safe, and effective trypanocidal agents (Baker *et al.*, 2013; Assefa & Shibeshi, 2018).

Natural products have historically played a crucial role in drug discovery, especially for parasitic diseases, due to their vast chemical and structural diversity (Njoroge *et al.*, 2014; Cheuka *et al.*, 2016; Dogara *et al.*, 2021). *Jatropha curcas* (barbados nut or physic nut), a plant readily available in Nigeria, is known for its multifaceted medicinal properties, including its use in traditional medicine (Chinwego, 2017; Jaspal *et al.*, 2023). Scopus search indicates that 5780 articles concern *Jatropha curcas*. This high number reflects that *J. Curcas* has, over time, attracted the attention of researchers and industrials for use as a biofuel and also a source of bioactive molecules. A quick bibliometric analysis may be necessary to cover the various studied areas, the best authors, and countries (Brika *et al.*, 2021; Laita *et al.*, 2024; Kachbou *et al.*, 2025; Verma *et al.*, 2025). The older one dated in 1829 treated "Experiments on the cassava root and on the juice of *J. curcas*" without any citation (Sonberiran, 1829). The most-cited paper (3572 times) is about improving lignin processing in the biorefinery (Ragauskas *et al.*, 2014). Concerning the country's ranking, Scopus analysis indicates that India dominates by more than 1300 articles, followed by India (850 articles.

This study, therefore, aimed to investigate the *in vitro* antitrypanosomal efficacy of the aqueous leaf extract of *J. curcas* against *Trypanosoma evansi* and to screen its phytochemical constituents, providing a scientific basis for its potential as a source of novel trypanocidal lead compounds.

Figure 1: *Jatropha curcas* leaves

2. Materials and methods

2.1. Plant material

Fresh leaves of *J. curcas* were collected from Zango, near the river, in Kaduna South Local Government Area, Kaduna State, Nigeria. The plant was identified and authenticated by botanist at the Department of Biological Sciences, Kaduna State University. A voucher specimen (No. 1232) was deposited at the University's herbarium. Fresh leaves of *J. curcas* were rinsed with distilled water, shade-dried at room temperature (25–30°C) for two weeks, and pulverized using a mortar and pestle in the department of biology Kaduna state university, Kaduna state (Yusuf *et al*., 2025; Sifawa, 2026).

2.2. Preparation of Extract

Five hundred grams (500 g) of the powdered material was macerated in 1000 mL of distilled water for 72 hours at room temperature with occasional agitation (Motsumi *et al.*, 2020; Nortjie, 2022). The mixture was filtered twice using muslin cloth. The filtrate was concentrated to dryness in a water bath at 40°C and then on a hotplate (40–60°C) to yield a solid residue. The crude aqueous extract was weighed, powdered, and stored in an airtight container at 4°C until use (Egbuna *et al.*, 2018; Alemba *et al*., 2024).

2.3. Phytochemical screening of the extract

The aqueous leaf extract was subjected to qualitative phytochemical screening to test for the presence of alkaloids, saponins, tannins, flavonoids, terpenes, steroids, cardiac glycosides, and glycosides according to standard procedures (Adekanmi *et al*., 2020; Khanal 2021; Nagori *et al*., 2025).

2.4. Test Organism

Trypanosoma evansi was obtained from stabilates maintained at the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna (Odeniran, 2020; Abdullahi *et al*., 2023). The parasites were maintained in the laboratory by serial passage in Wistar rats (Tijjani *et al*., 2020; Adebiyi *et al*., 2021). Blood with a high parasitaemia (approximately 10^7 parasites/mL) was harvested from a donor

rat via cardiac puncture at the peak of infection. The level of parasitaemia was determined microscopically (×400) using the rapid matching method (Herbert & Lumsden, 1976) (Bargul, 2015; Abera *et al*., 2016). The care and use of animals followed national and international guidelines (EEC Directive of 1986; 86/609/EEC) (Nakanishi, 20216; Olsson *et al*., 2017).

2.5. *In vitro* Antitrypanosomal Assay

A stock solution of the crude extract was prepared by dissolving 50 mg in 3 mL of culture medium (RPMI 1640 supplemented with 1% w/v D-glucose, pH 7.4) (Dore *et al*., 2014; Yamamoto *et al*., 2015; Bhushan *et al*., 2016). Serial dilutions were made with the culture medium to obtain final test concentrations of 20, 10, 5, and 2.5 mg/mL (Balouiri *et al*., 2016). Diminazene aceturate (Dimivet, SKM Pharma, India), a standard trypanocidal drug, was prepared at 2.5 mg/mL in the culture medium to serve as the positive control (Kamau, 2018).

The assay was performed in duplicates in a 96-well microtiter plate. For each well, 50 µL of the extract concentration or positive control was mixed with 30 µL of infected blood (containing 25–30 parasites per field) (Batiha *et al*., 2019; Batiha *et al*., 2020). The negative control well contained 30 µL of infected blood and 100 µL of culture medium only (Ledeboer *et al*., 2015; Vutukuru *et al*., 2016). The covered plate was incubated in a water bath at 37°C for 1 hour (Coffey, 2014). The *In vitro* antitrypasomal activity of the extract was expressed as percentage of inhibition (I %) using the formula described by Danjumma and Lawan, 2025.

$$\text{Percentage inhibition (\%)} = \frac{Control - Treated}{Control} \times 100$$

Where: Control (C) is the time taken for immobilization without treatment while treated (T) is the time recorded at each tested concentration.

2.6 Microscopic Examination

At 15-minute intervals (15, 30, 45, and 60 min), 2 µL of the mixture from each well was placed on a microscope slide, covered with a coverslip, and examined under a microscope (×400) for parasite motility (Bertiaux *et al* 2021; Jayawardhana, 2025). Motility was categorized as actively motile (++), sluggish/slightly motile (+), or non-motile (-). Complete cessation of motility in the treated samples, compared to the controls, was considered indicative of antitrypanosomal activity (Eke *et al*., 2017; Tauheed *et al*., 2020; Tauheed *et al*., 2022).

2.7 Data analysis

The data for this study was qualitative and simple descriptive statistics to interpret the experimental results.

3. Results and discussion

3.1 VOSviewer visualization

Scopus analysis can be visualized by VOSviewer representing countries by coloured nodes interconnected to show the international collaborations between countries (Elmsellem *et al.*, 2023; Judijanto *et al.*, 2024; Hammouti *et al.*, 2025; Foufa *et al.*, 2026). The network visualisation indicates more than 60 countries having at least 5 articles and more. India has the largest green node (1300 articles), compared to India (850 articles) shown by mustard node. The third position is Brazil (blue node) and the 4th country is Malaysia (brown node). The lines connecting the different countries represent collaborations on research projects, publications, or academic exchanges, with thicker lines likely indicating more frequent or more substantial collaborations.

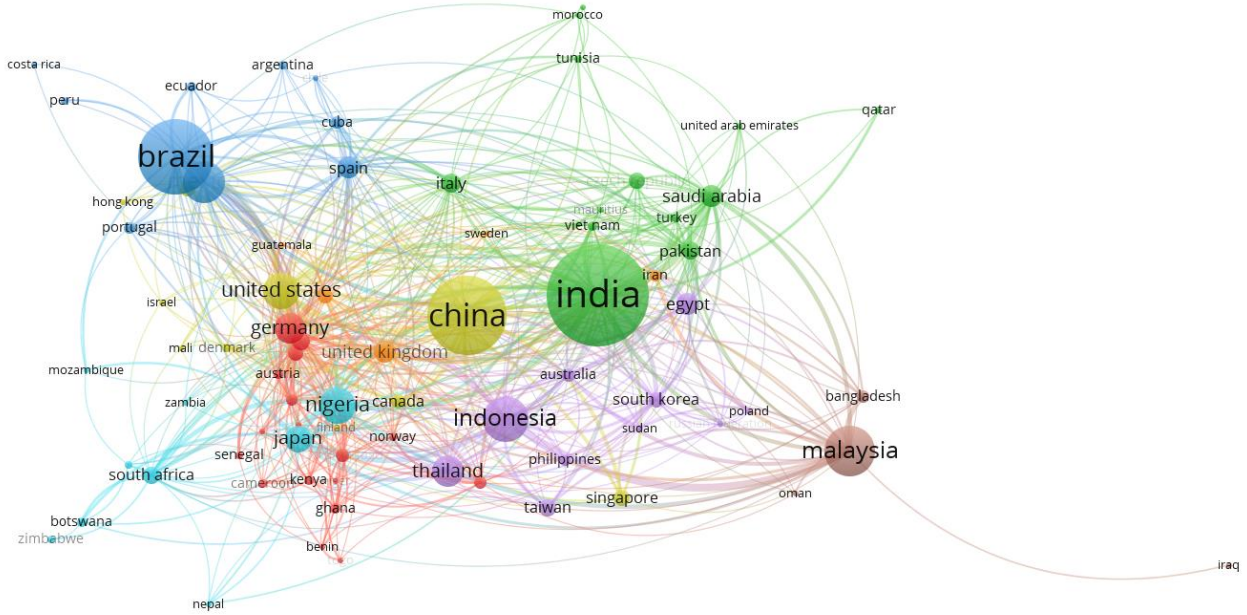

Figure 2: *Jatropha curcas* VOSviewer network visualization of countries (≥ 5 articles)

3.2. Phytochemical Constituents

The qualitative phytochemical screening from Table 1 detected the alkaloids (+), saponins (+), tannins (+), and cardiac glycosides (+) in the aqueous leaf extract of *Jatropha curcas*, while flavonoids, terpenoids and steroids were absent. Alkaloids are nitrogen-containing heterocyclic compounds known to interfere with parasitic microtubule formation and nucleic acid synthesis (Alamgir, 2018). Their presence is a strong predictor of antitrypanosomal activity. Saponins can increase cell membrane permeability via cholesterol complexation, potentially facilitating parasite lysis (Isah *et al.*, 2018; Shang *et al.*, 2025). Tannins (hydrolysable or condensed) bind to surface

proteins and enzymes of trypanosomes, inhibiting essential metabolic processes (Soto-Sànchez, 2022; Huang *et al*., 2024). Cardiac glycosides inhibit Na^+/K^+-ATPase; while primarily cardiotoxic in mammals, they may disrupt ion homeostasis in trypanosomes at sub-lethal concentrations (Obradovic *et al*., 2023; Huang *et al*., 2024).

Flavonoids and terpenes are often reported in organic solvent extracts (e.g., ethanol, methanol) but are poorly extracted by water due to their low polarity (Tzanova *et al*., 2020; Cheng *et al*., 2021). Thus, the negative results do not imply their absence in the plant, only that they are not present in sufficient concentration in the *aqueous* extract to be detected by the employed reagents (Morsy, 2014; Alamgir 2018). The combination of alkaloids, saponins, tannins, and cardiac glycosides in one aqueous extract is sufficient to exert membrane-active and metabolic-inhibitory effects on *Trypanosoma* species (Cock, 2015; Ungogo *et al*., 2020).

Table 1: Phytochemical Constituents of *J. curcas* Aqueous Leaf Extract

Constituent	Result
Alkaloids	+
Saponins	+
Tannins	+
Cardiac glycosides	+
Flavonoids	-
Terpenoids	-
Steroids	-

Key: (+) = Present; (-) = Not detected.

3.3. *In vitro* Antitrypanosomal Activity

Figure 3: Percentage inhibition of trypanosome immobilization at different concentrations of the extract and standard drug. The result shows a concentration-dependent increase in activity, with maximum inhibition observed at 20 mg/mL and the standard drug.

The in vitro antitrypanosomal activity of *J. curcas* extract against *T. evansi* was summarized in Table 2. The extract exhibited a clear concentration and time-dependent inhibitory effect on parasite motility. At the highest concentration (20 mg/mL), parasites became noticeably sluggish within 15 minutes and were completely immobilized by 30 minutes. At concentrations of 5 mg/mL and 10 mg/mL, complete immobilization was observed at 45 minutes, while the lowest concentration (2.5 mg/mL) achieved complete loss of motility at 60 minutes. The positive control, diminazene aceturate (2.5 mg/mL), demonstrated rapid trypanocidal activity, with parasites becoming sluggish within 15

minutes and fully immobilized by 30 minutes. In contrast, parasites in the negative control group remained actively motile throughout the 60-minute observation period. The highest concentration (20 mg/mL) produced 50% inhibition, which was comparable to the standard drug (DA). These findings demonstrate a dose-dependent antitrypanosomal effect of the extract.

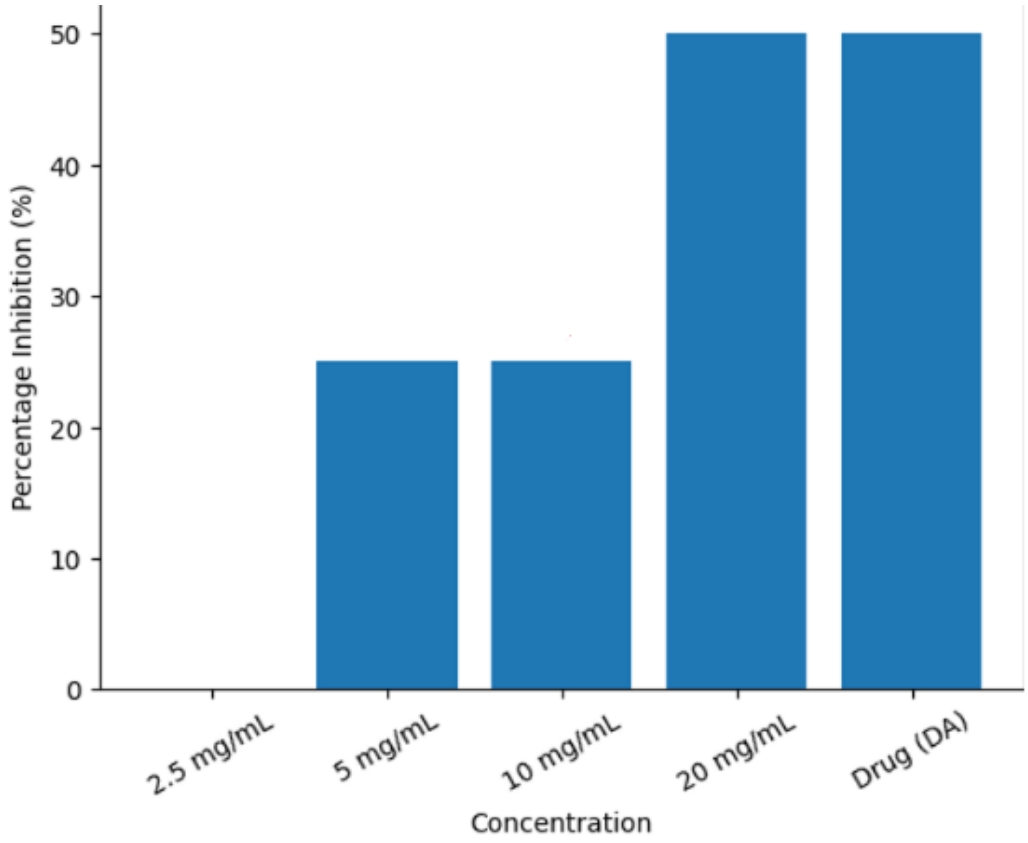

Figure 3: Antitrypasomal Activity (Percentage Inhibition)

Table 2: In vitro antitrypanosomal activity of *J. curcas* extract against *T. evansi*

Concentration (mg/mL)	Duration (minutes)			
	15	30	45	60
2.5	++	++	+	-
5	++	++	-	-
10	++	+	-	-
20	+	-	-	-
D.A (2.5)	+	-	-	-
Control	++	++	++	++

KEY: ++ =Actively motile, + =Sluggish**,** - =Non-motile and D.A= Diminazene aceturate (positive control).

The in vitro assay demonstrated that the aqueous extract of *J. curcas* exhibits mild, concentration-dependent antitrypanosomal activity against *T. evansi* (Dyary *et al*., 2014; Obeng *et al*., 2021). However, the observed efficacy was lower than that of the standard drug, diminazene aceturate, which

achieved complete parasite immobilization at the same concentration (2.5 mg/mL) within a shorter exposure time. The finding contrasts with the report by de Brito *et al*. (2020), which documented pronounced antitrypanosomal activity using a methanolic extract of *J. curcas*. The variation in activity may be attributed to differences in extraction solvents. Methanol, as a more versatile solvent, is capable of extracting a broader spectrum of phytochemicals, including both polar and non-polar compounds. This may result in the recovery of more potent bioactive constituents such as terpenoid and flavonoids, which were likely absent or present in lower concentrations in the aqueous extract used in this study (Lee *et al*., 2024; Ramesh *et al*., 2024). These observations highlight the importance of solvent selection in phytochemical extraction and its significant influence on the biological activity of plant-derived extracts. The observed antitrypanosomal activity, although mild, may be attributed to the phytochemical constituents identified in the extract. Notably, alkaloids, which were detected in the aqueous leaf extract of *Jatropha curcas*, are well recognized for their trypanocidal properties (Larayetan *et al*., 2019; Obeng *et al*., 2021). These compounds are reported to exert their effects through mechanisms such as interference with parasite DNA, enzymatic systems, and neurotransmission processes (MuKherjee *et al*., 2016; Idris *et al*., 2019).

The findings of this study revealed that the aqueous leaf extract of *J. curcas* exhibits mild in vitro antitrypanosomal activity against *Trypanosoma evansi*. This activity is likely associated with the presence of phytochemicals including alkaloids, saponins, and tannins. However, the extract showed lower efficacy compared to the standard drug, diminazene aceturate. This reduced activity may be due to the absence or low concentration of other potent bioactive constituents, such as flavonoids, steroids, and terpenoids, which are known to contribute significantly to antitrypanosomal effects.

Conclusion

Phytochemical screening of *J. curcas* aqueous leaf extract revealed the presence of fewer biological active compounds including tannins, saponins, alkaloids and cardiac glycosides; which can be responsible for the observed slightly antitrypanosomal activity of the plant in this study.

Conflict of Interest. The authors declare that there was no conflict of interest.

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