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In-vitro Antimalarial Activity and Phytochemical Screening of *Jatropha curcas* Leaf Extracts

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Abstract

The study was aimed at evaluating the antimalarial properties and phytochemical compounds present in the leaves of *Jatropha curcas Linn*. The extracts of the leaves of *J. curcas* were screened for the presence of secondary metabolites and in-vitro antimalarial activity against *Plasmodium falciparum* using the thin smear method. The results of the Bioassay all the fractions of *J. curcas* were found moderately active against the malarial parasite at concentrations of 5000, 2000, 1000, and 500 µg/ml with percentage elimination of 94.3, 92.9, and 91.4% respectively. The results indicated that the plant extracts contained an active compound(s) that have anti-malaria properties. The Phytochemical screening of the extracts revealed the presence of saponins, flavonoids, steroids, tannins and alkaloids. Therefore, the study justifies the use of leaves of *Jatropha curcas Linn* in traditional medicine for treating malaria fever in the Hausa community of Katsina State, Nigeria.

Keywords: Maceration; *J. curcas* Linn; phytochemistry screening; and *in-vitro*-antimalarial activity.

Introduction

Medicinal plants that produce and accumulate bioactive constituents provide a huge reservoir of various chemical compounds with potential therapeutic [1]. Despite the tremendous progress made in the development of drugs and antimicrobial agents, reported cases of occurrences of drug-resistant microbes and the emergence of unknown diseases causing microbes posed an enormous public health concern [2]. This fact forced the search for new antibiotics/antimicrobial compounds from various sources such as plants of medicinal origin to replace those plants that have become inactive [3]. Medicinal plants have been used as a source of medicine to treat illness since time immemorial which contributed immensely to human health [2]. The medicinal properties of plants depend largely on secondary metabolites that produce a definite physiological action on human beings [4]. These metabolites are usually found in various parts of the plants such as roots, leaves, shoots and stembarks [3]. The majority of local people in rural areas depend on herbal medicine for their drug needs. This could have been attributed to the prevailing economic conditions of people living in developing countries [5]. Bioactive compounds were reported to be present in medicinal plants for the pharmaceutical, cosmetics, and food industries, and in agriculture for pest control [6]. The use of crude traditional remedies in the form of water-based extracts, tinctures and concoctions was reported to be highest in individuals with diseases such as cancer, venereal, diabetes and arthritis [7].

However, about 80% of the African populace relies on orthodox medicine for their health care purposes, hence the need to further search for plants with medicinal remedy [8]. Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a type of unicellular microorganism) of the genus *Plasmodium*. The disease is commonly transmitted by a bite from an infected female Anopheles mosquito, which introduces the organisms from its saliva into a person's circulatory system [9]. The parasites travel in the blood to the liver to mature and reproduce. Malaria causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death [9]. Five species of *plasmodium* can affect and be transmitted by humans. The vast majority of deaths are caused by *P. falciparum* and *P. vivax* while *P. ovale*, and *P. malariae* which cause a generally milder form of malaria that is rarely fatal [9]. According to WHO 2019, the war against malaria fever is far from over. Perhaps it has been estimated in the previous year that about 228 million cases and 405,000 deaths occurred worldwide [10]. The lack of availability of licensed vaccine with fully protective required immunity, regulating the symptoms, occurrences and deaths by malaria could only be achieved using insecticide-treated bed nets and chemotherapeutic means at the present situation [11]. World Health Organisation (2024) reported that, in recent years, progress in reducing malaria has shown no improvement. Malaria continues to directly endanger health and cost lives, also perpetuates a vicious cycle of inequity. The most vulnerable people living in this situation including children under 5 years of age, pregnant women, infants, refugees, migrants, internally displaced people, and Indigenous Peoples continue to be disproportionately impacted [12].

Jatropha curcas L. (Euphorbiaceae) known as "Bini da Zugu'' in Hausa, is a large shrub 3-4m high, the leaves are alternately arranged 10-15cm x 7.5- 12.5 cm. They are broadly ovate, connate, acute usually palmate 3 or 5 lobed glabrous flowers in loose panicles of the cymes, yellowish green fruits are 2.5cm long avoid black seed are avoid-oblong, dull brownish black. The seed resembles castor seed in shape but are smaller in size and dark brown in colour [13],[16]. *J curcas* is most widely specie across different regions all over the world due to its strength. It has many names in different regions like the purging nut, physic nut, goat nuts, Barbados nut, nettle spurge, or just *Jatropha* [14]. However, previous research showed that *Jatropha* is native to Central and South America [15]. According to the literature, *Jatropha curcas* has been reported for its therapeutic potential. Both the leaves and the seeds were used in traditional medicine and were also reported to be effective in veterinary medicine. The leaves of *J. curcas* have been used for the treatment of coughs and as an antiseptic after birth [16]. The roots of *J. curcas* were used to treat pneumonia, syphilis, abortion and anthelmintic [17]. *J. curcas* had been considered a multipurpose plant. It was reported to be used in traditional human medicine and veterinary medicine for a long time [18]. However, it can decrease soil degradation, desertification, and deforestation [19]. Vegetable oil has a high capacity for removing waste from the body and is utilized as a remedy for skin diseases [17]. Different methods were established to transform vegetable oil such as *Jatropha* oil into biofuel like bio-kerosene [18]. Moreover, vegetable oil was extracted from the *Jatropha curca* L. seeds through chemical, mechanical and enzymatic processes [19]. The leaves of *J. curcas* were reported to contains cyclic triterpenes stigmasterol, stigmasterol-5-en-3, 7-diol, cholest-5-en-3,7-diol, campesterol and sitosterol, 7-keto-sistostorel, 7-keto-sitosterol as well as d-glucoside of sitosterol [20]. The roots part of *J. curcas* contains sitosterol and its d-glucoside, marmesin, propacin, the curculathyranes A and B and the curcusone A- D, diterpenoids jatrophol and jatropholone A and B the caumarin tomentin, the coumarin-lignan jatrophin and taraxerol [20]. The stem bark of *J. curcas* contains phytochemical constituents such as amyrin, sitosterol and taraxerol [20]. Thus, in this study, we report the *in-vitro* antimalarial activity and phytochemical screening of the ethanol extract, petroleum ether, chloroform, ethyl acetate,

methanol and aqueous fractions obtained from the leaves of *Jatropha curcas* Linn (Euphorbiaceae).

Materials and Methods

Plant Materials

The leaves of *Jatropha curcas* Lin were collected on 27th June 2023 from Tudun Katsira in Katsina Local Government Area of Katsina State Nigeria. The plant was identified at the Department of Biology Umaru Musa Yar'adua University Katsina.

Extraction and Fractionation

The air-dried leaves sample (100g) was percolated with 96% ethanol (400mL) at room temperature for two weeks. The crude ethanol extract was concentrated at 40°C under reduced pressure using a rotary evaporator (R110). The extract was weighed and labelled F001 [20]. The portion of ethanol extract (F001) was solvent partitioned between chloroform and distilled water (200mL, 1:1) with the aid of separating funnels. The Chloroform soluble fraction (F002), Aqueous soluble fraction (F003), and interface fractions between Chloroform and distilled water solvents F001* (where applicable), were separately concentrated to dryness on a rotary evaporator at 40°C whereas distilled water-soluble fraction, (F003) was washed several times with ethyl acetate (100mL), which yielded water-soluble fraction, (F003) interface fractions between distilled water and ethyl acetate solvents, (F002*), (where applicable), and ethyl acetate soluble fraction, (F004). A portion of the Chloroform residue obtained as mentioned earlier was transferred into a vial and kept for further analysis while the remainder were further portioned between methanol and n-hexane solvent (200mL, 1:1), were separated and concentrated to give methanol soluble fraction (F005), interface fractions between methanol and n-hexane, F003* (where applicable), and n-hexane soluble fraction (F006) respectively. All the fractions obtained were transferred into distinct vials and preserved.

Phytochemical Analysis of the Fractions

Phytochemical Analysis for quantitative detection of secondary metabolites was performed on the fractions obtained as described by Harbone and Evans [21], [22].

Test for alkaloids

Each fraction (0.5g) was dissolved in 5ml of 5% ethyl ether and stood for 15 minutes. The sample was extracted for 2.0 minutes with 5ml of aqueous HCl on a boiling water bath. The resulting mixture was centrifuged for a few minutes at 3000rpm. 1ml of the filtrate was treated with few drops of Mayer's reagent and a second 1ml with Dragendroff's reagent and turbidity was observed [21], [22] & [23].

Test for anthraquinones

Each fraction (0.5g) was shaken with 10ml of benzene and filtered. 0.5ml of 10% ammonia was added to the filtered and the mixture was shaken well and the presence of the violet colour in the layer phase indicates the presence of the anthraquinones [21], [22] & [23].

2.3.3. Test for flavonoids

A portion of each fraction was heated with 10mls of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4mls of the filtrates were shaken with 1ml of dilute ammonia solution and yellow colouration was observed.

Test for glycosides

Each fraction (0.5g) was dissolved in 5 ml of methanol. 10 ml of 50% HCl was added to 2 ml of methanol extract in a test tube. The mixture was heated in a boiling water bath for 30 minutes. 5ml of Fehling solution was added and the mixture was boiled for 5 minutes to observe a brick-red precipitate as an indicator for the presence of glycosides [21].

Test for phlobatannins

A portion of each fraction was boiled with 1% aqueous HCl acid to observe the deposition of

red precipitate is an indication of the presence of phlobatannins.

Test for saponins

A portion of each fraction (0.5 g) was shaken with water in a test tube and it was warmed in a water bath and the persistence of froth indicates the presence of saponins.

Test for steroids

A portion of each fraction (0.5 g) was dissolved in 5 ml of methanol. 1 ml of the extract was treated with 0.5 ml of acetic acid anhydride and was cooled in ice. This was mixed with 0.5 ml of chloroform and 1 ml of concentrated sulphuric acid was then added carefully using a pipette.

Test for tannins

A portion of each fraction (0.5 g) was stirred with 10 ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as evidence of the presence of tannins [21]

Test for terpenoids

A portion of each fraction (0.5 g) was dissolved in 5 ml of methanol. 2 ml of the fraction was treated with 1 ml of 2,4-dinitrophenyl hydrazine dissolved in 100 ml of 2M HCl. A yellow-orange colouration was observed as an indication of terpenoids.

MALARIA PARASITE BIOASSAY

Preparation of test solution

A stock solution of 10,000µg/ml was prepared by dissolving 20 mg of each test fraction 2ml of Dimethyl sulphoxide (DMSO). The sample solutions of 500, 1000, 2,000, 5,000 µg/ml were prepared from the stock solution by serial dilution [1].

Sourcing of Malaria parasite for Assay

Parasites of infected blood samples containing *Plasmodium falciparum* were collected from the Department of Haematology, Umaru Musa Yar'adua University Clinic. The samples were received in K3-EDTA coated disposable plastic sample bottles with tightly fitted plastic corks, and transported to the Biology Laboratory of Umaru Musa Yar'adua University.

Separation of the Erythrocytes (5% parasitaemia) from Serum of Blood samples

In the method employed by Dacie and Lewis, 50% dextrose solution (0.5ml) was added to each blood sample (5ml) which was defibrinated and centrifuged at 2500 rpm for 15 minutes in a spectra merlin centrifugation machine. Blood samples with higher parasitaemia (>5%) were diluted with fresh malaria parasite-negative erythrocytes [24].

Preparation of *Plasmodium falciparum* culture medium

The venus blood (2ml) from the main vein of a white healthy rabbit pinnae was collected using a syringe (BD 205WG). This was defibrinated and allowed to settle for at least 45 minutes [25]. The blood was then centrifuged. The sediments were discharged and the serum collected was supplemented with RPMI 1640 salt medium (KCl 5.73 mM, NaCl 10.27 mM, $MgSO_4$ 2.56 mM, $NaHPO_4$ 17.73 mM, $Ca(NO_3)_2$ 0.42 mM, $NaHCO_3$ 2.5 mM and Glucose 11.0 mM (BDH Ltd, UK) [26]. The medium was sterilized by 50 gentamicin sulphate [27].

***In vitro Assay* of the Activity of the extract on *Plasmodium falciparum* culture**

The assay was performed using RPMI 1640 as the culture medium used for cultivation of *P. falciparum* [28]. Controls were prepared without the plant extracts. Each test solution (0.1 ml) and the culture medium (0.2 ml) was added into test tubes containing 5% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to each tested fraction at concentrations of 500, 1000. 2000 and 5000µg/ml was determined microscopically at 37°C after 24 and 48 hours of incubation. The incubation was undertaken in a glass bell jar containing a lighted

candle to ensure the supply of the required quantity of CO_2 (about 5%) O_2 gas 2% and about 93% nitrogen gas [29].

3.4.2 Determination of the activity

After 24 and 48 hours of incubation, an aliquot of the culture medium was dropped on a microscopic slide and strained using Giemsa's staining techniques. The average percent elimination of the erythrocytes that appeared as blue discoid cells was determined using the formula.

$\%A = N/N_x \times 100$

Where % A = Percentage activity of the extracts

N = Total number of cleared Red Blood Cells (RBC)

N_x = Total number of parasitized RBC

3. Results and Discussions

The leaves of the plant were percolated with ethanol yielding a crude extract. The percolation of the crude extracts with solvents of different polarity gives several fractions (Table 1). The amount and appearance of each fraction are presented in (Table 1). The results of phytochemical analysis revealed that alkaloids, flavonoids, glycoside, steroids and terpenoids are present in all the fractions (Table 2). Whereas saponins were observed only in the ethanol extracts. Tannins and phlobat tannins were observed in both ethanol and methanol soluble fractions. The *in-vitro antiplasmodial* activity reflects the effectiveness of each sample fraction in determining the elimination of the number of the parasitized cells in the test culture after 24 and 48 hours' incubation period (Table 3). Among the sample fractions tested, ethanol and chloroform fractions recorded 94.3% elimination of the parasites. This was followed by chloroform soluble fraction with percentage activity of 92.9% elimination ability at 5000 µg/ml respectively. Ethyl acetate fraction demonstrated fair elimination of parasites with a percentage activity of 55.2 % elimination ability at 5000 µg/ml. Similarly, a weak elimination <50% has been observed from ethyl acetate fraction at 2000, 1000 and 500 µg/ml respectively. It was also observed that the activity of the extracts increases with an increase in concentration.

Table 1: Physical characteristics of fractions obtained from *Jatropha curcas* leaves extracts

Fractions	Code	Weight(g)	Texture	Appearance
Ethanol	F001	10.92	Gummy	Dark green
Petroleum ether	F002	2.32	Sticky	Dark green
Chloroform	F003	2.02	Sticky	Dark green
Ethyl acetate	F004	2.98	Sticky	Dark green
Methanol	F005	2.00	Gummy	Dark green
Aqueous	F006	1.05	Gummy	Dark green

Table 2: Results of Phytochemical screening

Plant (part)	Fraction	Phytochemicals						
		Alk	Fla	Gly	Phl	Sap	Ste	Tan
Ter								
leaves	F001	+	+	+	+	+	+	-
+								
	F002	+	+	+	-	-	+	-
+								
	F003	+	+	+	-	-	+	-
+								
	F004	+	+	+	-	-	+	-
+								
	F005	+	+	+	+	-	+	-
+								
	F006	+	+	+	-	-	+	+
+								

Key: *Alk = alkaloid, Fla = Flavonoids, Gly = Glycoside, Phl = Phlobat tannis, Sap = Saponins, Tan = Tannis Ter = Terpenoids. F003 = ethanol extract; F003 = chloroform fraction; F004 = ethyl acetate fraction + = positive, - = negative*

Table 3: Anti-plasmodial activity Results of *Jatropha curcus* Leaves Fractions

Fractions	Conc. µg/ml	Average no. of parasite per field before incubation	Overall average no. of parasite after 48 hours	Total cleared RBC	Percentage of elimination at the end of incubation (%)
F001	500	35	02	33	82.9
	1000	35	06	29	88.6
	2000	35	04	31	91.4
	5000	35	03	32	94.3
F002	500	35	03	32	68.6
	1000	35	11	24	86.6
	2000	35	03	32	91.4
	5000	35	02	33	94.3
F003	500	42	03	39	61.9
	1000	42	11	31	73.8

	2000	42	16	26	76.2
	5000	42	10	32	92.9
F004	500	29	16	13	44.8
	1000	29	16	13	44.8
	2000	29	15	14	48.3
	5000	29	13	16	55.2
F005	500	40	02	38	82
	1000	40	06	34	85
	2000	40	02	38	89
	5000	40	03	37	90

Conclusion

The present research work demonstrated that the extracts of the leaves of *Jatropha curcas* have good activity against the *plasmodium* parasite particularly the ethanol (F001) and chloroform fractions (F002). Thus, the results obtained in this work established the efficacy of the plant extracts used in traditional medicine for the treatment of malaria. The information obtained from this study indicated that further investigations need to be directed towards isolation, purification, characterization and testing of the *in-vitro* anti-plasmodial activity against the pure compound obtained from the leaves of the plant. This task is currently underway in our research laboratory.

Conflict of interest

The author has no conflict of interest to declare.

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