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PHYTOCHEMISTRY AND ANTIPLASMODIAL PROPERTIES OF AQUEOUS AND METHANOL LEAF EXTRACTS OF *Jatropa curcas*

Imam, A. A.,[1*] Salim, M.A.,[2] Bala, M.,[1] Aisha, M. I.[3] and Yahaya, S.[3]
[1]Department of Biochemistry, Faculty of Biomedical Sciences, Bayero University Kano, P.M.B3011 Kano-Nigeria
[2]Departmet of Human Physiology, Faculty of Biomedical Sciences, Bayero University Kano, P.M.B3011 Kano-Nigeria
[3]Department of Microbiology, Faculty of Science, Bayero University Kano, P.M.B3011 Kano-Nigeria
[*]Corresponding Author: aaimam.bch@buk.edu.ng; 08146587087

ABSTRACT
This study was aimed at evaluating the phytochemistry and antiplasmodial properties of the aqueous and methanolic leaf extracts of Jatropa curcas against the malaria parasite; Plasmodium falciparum. The need for new therapeutic compounds against malaria parasite is made more urgent by the continued spread of resistance to almost all the available anti malarial drugs. Phytochemical detection followed by antiplasmodial sensitivity test was carried on the two leaf extracts following standard procedures. The pyhtochemicals detected from both extracts were alkaloids, glycosides, tannins, saponins, phenol, steroids and flavonoids. The result of Plasmodium sensitivity tests revealed that both the aqueous and methanolic leaf extracts of Jatropa curcas were highly effective against the malaria parasite. However, the methanolic extract showed greater activity than the aqueous extract. At extract concentration of 10mg/ml, the methanol extract produced the highest parasite clearance rate after 72 hour incubation period, with percentage elimination of 95.4% while the aqueous extract produced 87% elimination at the same concentration and incubation period. These observations showed that Jathropa plant may contain chemicals with promising antimalarial properties which when fully harnessed could constitute a novel therapy for the management of clinical malaria.
Keywords: Antiplasmodial, Jatropha Curcas, Extracts, Phytochemistry.

INTRODUCTION

Malaria is one of the world's most deadly diseases. Even though it is highly preventable and treatable, it causes approximately 881,000 deaths every year, with nine out of ten deaths occurring in sub-Saharan Africa, and 85% of malaria-related deaths in children under five years of age. This is the equivalent of a child dying of malaria in Africa every 30 seconds. The most serious forms of the disease are caused by the parasite *Plasmodium falciparum*; malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* results in milder disease in humans that is not generally fatal. In 2008, about 109 countries were reported to be endemic for malaria, with 45 of the countries within the African region. There were an estimated 247 million episodes of malaria in 2006, with 86% of cases reported in African countries (Kokwaro, 2009).

Malaria is mostly a disease of hot climate. The Anopheles mosquito, which transmits the malaria parasite from one human being to another, thrives in warm, humid climates where pools of water provide perfect breeding grounds. It proliferates in conditions where awareness is low and where health care systems are weak (UNICEF, 2000). Malaria is unique among diseases because its roots lie so deep within human communities. The most dangerous vectors of malaria thrive mainly within the village and sub-urban environments. Logically, the adult vectors remain close to their nocturnal source of human blood and the developmental stages of these mosquitoes exploit the nearby accumulations of water that form where people have disturbed the natural drainage. Malaria then becomes a fixture of village life, exacting a continuous toll on the health of its host population while generating lethal outbreaks among visitors (Heggenhougen *et al.,* 2003).

Malaria is a life-threatening blood disease caused by the plasmodium parasites and is transmitted to humans by the *Anopheles* mosquito. Once bitten, parasites multiply in the host's liver before infecting and destroying red blood cells. Derived from the Italian word for "bad air," it was originally thought swamp fumes in Rome were the cause of malaria, as outbreaks were a regular occurrence there.

There are more than 100 types of *Plasmodium* parasites, which can infect a variety of species (WHO, 2013). Scientists have identified five types that specifically infect humans, they are: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi* . Plasmodium falciparum is the most common of these species and is responsible for more than 80% of incidence of clinical malaria (WHO, 2013).

The development and spread of parasite resistance to certain anti-malarial agents has presented a major barrier to successful disease management in malaria-endemic areas and has probably contributed to the resurgence of infection and the increase in malaria-related deaths in recent years. Resistance to almost all commonly used anti-malarials, notably chloroquine and sulphadoxine-pyrimethamine, but also amodiaquine, mefloquine, and quinine, has been observed in the most lethal parasite species, *P. falciparum* (Kokwaro, 2009). The problem of resistance exists in much of Africa and Southeast Asia; for example, treatment failure rates of around 70-80% have been reported for chloroquine (Vestergaard and Ringwald, 2007), which was formerly the cheapest and most widely available anti-malarial drug. There is also increasing drug resistance to amodiaquine in large parts of East Africa, potentially rendering the combination of artesunate and amodiaquine less effective.

Using anti-malarials that have evolved from similar basic chemical compounds can lead to an increase in the development of resistance. For example, the relatively high rate of treatment failure reported with the ACT dihydroartemisinin-piperaquine against *P. falciparum* may be attributed to cross-resistance between chloroquine and piperaquine (Karunajeewa, 2008). This may reduce the usefulness of new therapies prior to large-scale deployment if they are derivatives of currently used drugs to which resistance has already been established.

Artemisinin derivatives are extremely potent anti-malarials with a rapid onset of action and when administered in combination with anti-malarial drugs with slower elimination rates (e.g. lumefantrine), short courses of treatment (three days) have proved to be highly effective. The less effective single-drug treatments increase the chance of parasites evolving and becoming resistant to the treatment; combining anti-malarial drugs with independent modes of action can impede the development of resistance to each individual component of the combination. In the rare event that a mutant parasite resistant to one of the drugs arises during the course of an infection, the parasite will be killed by the other drug in the combination (Kokwaro, 2009).

For a long time, plants have provided a source of emerging modern medicines and drug compounds, as plant derived medicines have made large contributions to human health. Medicinal plants like *Jatropha curcas* have played major role in the treatment of various diseases, including bacterial and fungal infections. All parts of *Jatropha* (seeds, leaves, bark, etc) have been used in traditional medicine (TM) and for veterinary purposes for a long time. Extensive public interest and expansion in the use of herbal medicine have led to new emphasis and drive in medical plant research. The research approaches taken recently include activities to develop herbal medicines into quality, efficacious and safe products for human consumption. This can be an advantage for *J. curcas* for its potential to be expanded as a herbal medicine to cure many illnesses and diseases. The cure for these illnesses and diseases lies in the chemical compositions isolated from different parts of the plant (Prasad *et al.*, 2012). Traditional medicines have been used to treat malaria for thousands of years and are the source of the two main groups of modern antimalarial drugs (artemisinin and quinine derivatives). The usual reason for interest in TM stems from the recognition that two of the major antimalarial drugs trace their origin to traditional medicines. The development of traditional medicines for malaria and modern drug development are not mutually exclusive. The development of resistance to virtually all known antimalarial drugs including the once highly effective ACT- based therapies has necessitated the need for development of new therapeutic compounds for malaria management and since plants has always provided many of the past effective therapeutic compounds, it is necessary to screen and study many plant species for novel and more effective therapy for malaria. Hence this study.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Jatropa curcas* were collected at Na'ibawa, Kumbotso Local Government Area, Kano State, in September, 2015. Identification was confirmed by a Botanist at the Department Biological Sciences, Bayero University, Kano. The Herbarium Accession number of the specimen is BUKHAN 0060.

Extraction of plant leaves

Aqueous and methanol extracts of the leaves of the plant were prepared according to the standard method described by Veeramuthu *et al.*, (2006). The plant samples were air dried and ground to powder using sterile mortar and pestle. The powdered material (100g each) was dissolved in 1000ml of distilled water and methanol respectively. Methanol (polarity index 5.1) and aqueous (water polarity index 9.0) extracts were obtained using percolation method and then filtered. The aqueous extract was concentrated to dryness using a freeze drier while the methanolic extract was concentrated in a rotary evaporator and allowed to dry at ambient temperature. The residues obtained were transferred into pre-weighed sample containers and stored at room temperature before use.

Phytochemical Analyses

Screening and quntification of the phytochemicals; alkaloids, tannins, flavonoids, saponins, glycosides, phenols, terpenoids and steroids were carried out using the standard procedures described by Harborne (1973) and Trease and Evans (1989).

Malaria Parasite Assay

Sourcing of Malaria Parasites for the Assay

Malaria parasites infected blood samples from the Clinic, Bayero University, Kano provide clinical blood samples containing heavy parasitemia of *Plasmodium falciparum*. Venous blood from patients recommended for malaria parasites test (MP test) was obtained using 5ml disposable plastic syringes and needles. The samples were immediately transferred into K3-EDTA disposable plastic sample bottles with tightly fitted plastic corks, mixed thoroughly and then transfered to the Microbiology laboratory at Bayero University, Kano in a thermoflask containing water maintained at 4°C.

Confirmation of *Plasmodium falciparum* positive blood samples using thin smear

After thorough mixing, a small drop of each blood sample was placed at the centre of a clean grease-free glass slide, at least 2mm from the edge using a clean capillary tube. A clean cover slip was placed in front of each drop at an angle about 45° and then drawn backward to be in contact with the drop of blood, the drop was then allowed to run along the full length of the edge of the cover slip. With a fast and smooth movement, the cover slip was pushed forward to form even thin smear on each glass slide. The smear was left to air dry and then stained using the leishman's stain. The stained slide was allowed to air dry and then observed under a high power objective (×100) using oil immersion. The smears were screened thoroughly for *Plasmodium falciparum* infected RBC's. An average parasitemia was obtained from the reading of 3-5 microscopic fields (Hanne *et al.*, 2002). Blood samples with 5% parasitemia were used for the research.

Separation of the Erythrocytes

About 5ml of Blood sample with 5% parasitemia was centrifuged at 2500 rpm for 15mins. After centrifugation, the supernatant (plasma) was discarded while the sediments (erythrocytes) were further centrifuged with normal saline at 2500 rpm for 5mins. The supernatant was then discarded and the erythrocytes were suspended in normal saline.

Preparation of *Plasmodium falciparum* Culture Medium

The media was prepared by dissolving 10.4g of the powdered material into one liter of distilled water and then autoclaved at 121°C for 15mins as instructed by the manufacturers. Venous blood (2ml) from the main vein of white healthy rabbit's pinnae was withdrawn using a disposable 5ml syringe (BD 205 WG). This was defibrinated by allowing it to settle for at least one hour. The defribranated blood was centrifuged at 1500rpm using spectre merlin centrifuge for 10mins and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500rpm for 5mins and the supernatant layer was added to the first test tube. The sediments were discarded and the serum collected was supplemented with the salt of RPMI 1640 medium (KCl 5.37mM, NaCl 10.27mM, MgS04 mM, NaHPO4 17.73mM, Ca(NO3)2 0.42mM, NaHCO3 2.5mM, and glucose 11.0 mM. (BDH ltd, UK). The medium was sterilized by 40µg/ml gentamicin sulphate (Trager, 1982).

Preparation of the Test Concentrations

An electronic digital balance, model (FA2104A) Gulfex Medical and Scientific Company, England, was used to measure 20mg of each of the extracts and then dissolved in 1ml of dimethylsulphoxide (DMSO) in separate vials (stock solution). Using serial doubling dilution, four different concentrations (10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml) of each extract were prepared.

***In-Vitro* Assay Of the Activity of the Extracts on *Plasmodium falciparum* Culture**

Exactly 0.1ml of test solution and 0.2ml of the culture medium were added into a tube containing 0.1ml of 5% parasitemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to the tested fractions was determined microscopically after incubation for 24, 48 and 72 hours at 37°C. The incubation was undertaken in a bell jar glass containing a lighted candle to ensure the supply of required quantity of Carbon dioxide (about 5% Oxygen gas, 2% and about 93% nitrogen gas as demonstrated by Muktar *et. al* (2006).

Determination of Antimalarial Activity

At the end of each incubation period, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Leishman's staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasite (that appeared red pink) was estimated and the average percentage elimination by the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites after each incubation period, using the formula below;

$$\% = \frac{N}{Nx} \times 100$$

Where, % = Percentage activity of the extracts
N = Total number of cleared RBC
Nx = Total number of parasitized RBC

Note: RBC = Red Blood Cells (Muktar *et al.*, 2006).

Statistical Analysis

Values are expressed as mean ± standard error of mean (SEM). Data was analyzed by ANOVA using the SPSS statistical package (Version 20).

RESULTS AND DISCUSSION

Table 1 blow shows the physical properties of the aqueous and methanolic leaf extracts obtained. Exactly 100g of the plant leaves were extracted with water and methanol respectively. The aqueous extraction yielded 19.01g of the extract which appeared powdery and brown in color. The methanol extraction yielded 16.44g of extract with a dark green color and gummy in texture.

Table 1: Physical Properties of Leaf Extracts from *Jatropa curcas*

Property	Aqueous extract	Methanolic extract
Weight of plant extract (g)	100	100
Weight of Extract (g)	19.01	16.44
Percentage Yield (%)	19.01	16.44
Colour of Extract	Brown	Dark green
Texture of Extract	Powdery	Gummy

The results of phytochemical analysis showed the distribution of the secondary metabolites in the extracts. From table 2, both aqueous and methanolic extracts have shown the presence of tannins, saponins, glycosides, steroids, alkaloids and phenol. Flavonoid was present in the methanolic extract but absent in the aqueous extract. Both the extracts did not reflect the presence of terpenoids.

Table 2: Phytochemical Constituents of *Jatropa curcas*

S/N	Phytochemical	ME	AE
1	Tannins	+	+
2	Saponins	+	+
3	Glycosides	+	+
4	Flavonoids	+	−
5	Steroids	+	+
6	Alkaloids	+	+
7	Tarpenoids	−	−
8	Phenol	+	+

Key: ME= Methanolic extract, AE= Aqueous extract, + (positive), − (negative)

The results of antimalaria activity of the extracts are shown in Table 3. Both the aqueous and methanolic leaf extracts have demonstrated a remarkable activity at all concentrations. The highest antiplasmodial activity was obtained with methanolic extract, in which the microscopic examination of stained slides at 10mg/ml showed a virtual absence of the parasite after 72 hours with percentage elimination of 95.4%. The least antiplamodial activity was observed with the aqueous extract at a concentration of 1.25mg/ml yielding a percentage elimination of 80%. Figures 1 & 2 showed initial and final parasite population structure after 72 hours exposure to the methanolicextract.

Table 3: Antimalarial Activity of *Jatropa curcas* Leaf Extracts with Percentage Eliminations after 72hrs Incubation

Solvent	Concentrations (mg/ml)	Average no. of parasites before incubation	Average no. of parasites during incubation			Average no. of parasites after incubation	Percentage elimination at the end of incubation
			24h	48h	72h		
	Positive control	13	1	0	0	0.3	98%
	Negative control	13	20	21	21	21.0	0%
	10.0	13	1.1±0.2		0.6±0.6	0.6 ± 0.6	95.4%
			0.0±0.0				
Methanol	5.00	13	2.3±0.6		1.0±0.0	1.4 ± 0.8	89.2%
			1.0±0.0				
	2.50	13	2.7±0.6		1.3±0.6	1.6 ± 1.0	88%
			0.7±1.2				
	1.25	13	3.0±1.0		1.3±0.6	1.9 ± 0.9	85.4%
			1.5±0.7				
	10.0	13	3.0±0.0		1.0±1.0	1.7 ± 1.2	87%
Aqueous	5.00	13	1.0±0.0			1.8 ± 1.1	86.2%
	2.50	13	3.0±1.0		1.3±0.6	2.3 ± 1.2	82.3%
	1.25	13	1.0±0.0			2.6 ± 1.0	80%
			3.7±1.2		1.7±0.6		
			1.5±0.7				
			3.7±0.6		2.0±0.0		
			2.0±1.0				

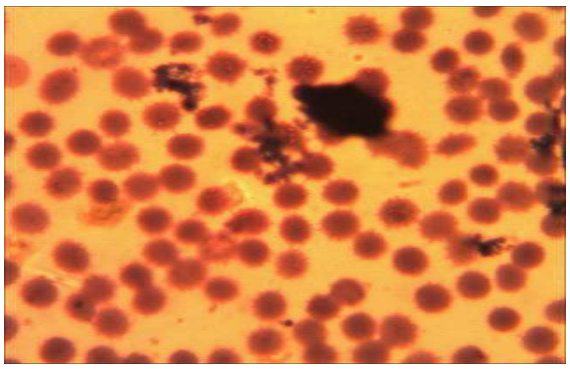

Fig. 1: Initial Parasite count before incubation with methanol extracts

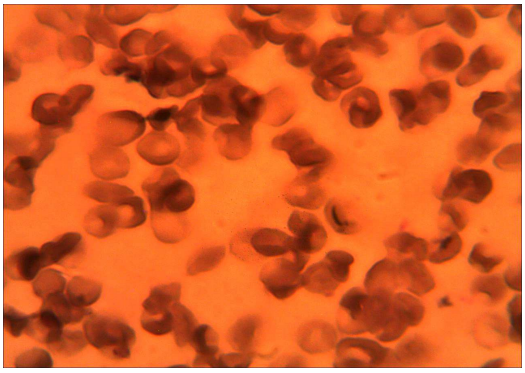

Fig. 2: 72 Hours after incubation with methanol extract

DISCUSSION

The phytochemical screening of the leaf extracts of *J. curcas* (Table 2) showed the distribution of the presence of different secondary metabolites in the extracts. Phytochemicals are compounds that occur naturally in plants, they have biological significance but are not established as essential nutrients. Both aqueous and methanolic leaf extracts indicated the presence of almost all the tested secondary metabolites with the exception of flavonoids which was only present in the methanol extract and terpenoids were found to be absent in both extracts. These variations could be attributed to the differences in the polarity index of the solvents. Solvents are classified into polar and non polar. Generally, the dielectric constant of a solvent provides a rough measure of its polarity. Polarity of a solvent is attributed to its attraction power to different compounds in a solution. The substances in a solution interact with each other at a molecular level, polar solvents like water attract polar compounds like salts and sugars while non polar solvents attract non polar compounds. Methanol with polarity index of 5.1 is known to extract both polar and non polar compounds. Basically, the choice of solvent relies on the polarity of the compounds of interest present in the plant material.

Phytochemical compounds such as alkaloids are commonly implicated in the antiplasmodial activity of many plants (Okokon *et al*., 2006). Some alkaloids targets the plastid-like organelles of the parasite called apicoplast, while some inhibit parasitic protein synthesis. Flavonoids revealed significant anti-parasitic activities against different parasite strains of malaria, trypanosome and leishmania (Al-Adhroey *et al*., 2010). The exact mechanism of anti malarial action of flavonoids is unclear but some are shown to inhibit influx of L-glutamine and myoinositol in infected erythrocytes. The bioactive compounds which were found in these extracts may be acting singly or in synergy with one another to exert the observed antiplasmodial activity of the leaves of *Jatropa curcas*.

The method of percolation was used in the extraction of the fresh leaves of *J. curcas.* Although there was a slight difference in the phytochemical screening, both the aqueous and methanol extracts showed a remarkable antiplasmodial activity with great percentage eliminations. The result revealed that both the aqueous and methanolic leaf extracts of *Jatropa curcas* exhibited some anti malarial activity. Even though the antiplasmodial activity of the methanolic leaf extracts was seen to be higher, however, there is no statistical significant difference in the activity of the two extracts, (P=0.002).

At 10mg/ml, the methanolic extract showed almost virtual absence of the parasites at the end of the incubation period, with percentage elimination of 95.4% while the aqueous extract yielded 87% elimination at the same concentration. The least activity which was also significant (P<0.002) was observed with aqueous extract at 1.25mg/ml which yielded a percentage elimination of 80%. These observations suggest that the activity of the extracts may be cytotoxic for the malaria parasite, there by inhibiting their growth and development.

From Table 4.3, it can be seen that the average number of parasites increased as the concentration of the extract decreased. This indicates that higher concentrations of the extracts were found to be more effective on the parasites. However, the methanolic extracts showed higher elimination than the aqueous extracts, the elimination rate for both the extracts was seen to be rapid within the first 24hrs, which then goes steady within the next incubation periods.

Contribution of Authors

Author Imam, A.A. supervised the research work and wrote the first draft of the manuscript. Author Yahaya, S. co-supervised the research work and made corrections to the manuscript. Author Aisha, M.I. performed some of the laboratory work while authors Salim, M.A. and Bala, M. made extensive corrections to the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest for this manuscript.

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