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PHYTOCHEMISTRY AND *IN VITRO* ANTIPLASMODIAL PROPERTIES OF AQUEOUS AND ETHANOL STEM BARK EXTRACTS OF *Jatropha curcas* (Physic Nut)

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ABSTRACT
The increasing prevalence and distribution of malaria is due to a number of factors, one of which is the emergence and spread of drug resistant parasites. Efforts are now being directed towards the discovery and development of new chemically diverse antimalarial agents. The present study reports on the phytochemistry and in vitro antiplasmodial properties of aqueous and ethanol stem bark extract of Jatropha curcas. Maceration method of extraction was used. The two extracts were assayed at various concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml for antiplasmodial activity against Plasmodium falciparum and were also screened for the presence of phytochemicals following standard procedure. The phytochemicals detected from both extracts were alkaloids, cardiac glycosides, anthranol glycosides, saponins, phytosterols, phenols, flavonoids, protein and amino acids and diterpenes. The result of antiplasmodial activity revealed that both the aqueous and ethanol stem bark extracts of Jatropha curcas were effective against the malaria parasite. However, the aqueous extract showed statistically (P<0.05) higher activities than the ethanol extract. At extract concentration of 10mg/ml, both the ethanol and aqueous extracts produced the highest parasite clearance rate after 72 hours incubation period, with percentage elimination of 77%. From these observations, Jatropha curcas is likely to contain promising chemical compounds which can be utilized as an effective plant-based chemical therapy for the treatment of malaria.
Keywords: Antiplasmodial, Jatropha curcas, Phytochemistry, Plasmodium falciparum,

INTRODUCTION

Plants extracts used as traditional medicine continues to provide for over 80% of the world's population health coverage, especially in the developing world (Igbinosa *et al.,* 2009). Phytochemicals (plant derived compounds) are natural alternatives to synthetic compounds which have been attracting much interest. The treatment of various diseases are carried out by using extracts of plants and this forms the basis for all traditional systems of medicine (Mbakwem-Aniebo *et al.,* 2012).

The word *Jatropha* is derived from a Greek word *Jatrus* meaning Doctors and Trophe which means Nutritional food (Aina *et al.,* 2016). *Jatropha curcas* or physic nut is a non-edible multipurpose shrub belonging to the family *Euphorbiacea*. It is an uncultivated non-food wild-species which grows in tropical and sub-tropical regions of the world (Lasalita-Zapico *et al*., 2012). Different parts of the plant have been reported to contain phenolics, flavonoids, saponins, glycosides, tannins and alkaloids. And these phytochemicals are said to have potentials as antimicrobial, anti-inflammatory, anticancer and antioxidant principles (Oloyede *et al*., 2012).

The extracts from different parts such as root, stem bark and leaves of *J. curcas* plant have been used in ethnomedicine (Aina *et al.,* 2016). Stems of young leaves have been used to successfully treat urinary infections. The roots are used to make an antidote for snake bites, decoction as mouth wash for bleeding gums and tooth ache as well as for eczema, scabies and ringworm. Tea from the bark of *J. curcas* is given to people with leprosy and rheumatism. The seeds contain between 27% to 40% oil which can be processed to produce a high-quality biodiesel fuel usable in a standard diesel engine (Agbogidi *et al.,* 2013).

Malaria is one of the most serious pathogenic diseases in endemic areas of the world, particularly in Africa, Asia, and Latin America. In Africa alone, it is estimated that more than 300 million people are infected annually by the parasite, *Plasmodium falciparum*, and over one million deaths have been recorded in children less than five years (Omoregie and Sisodia, 2012). Malaria control efforts are having a positive impact, thus contributing to the declining global prevalence of malaria in recent years (Chinsembu, 2015).

Control of malaria is still one of the world's chief current public health challenges, particularly in sub-Saharan Africa where it is still responsible for 10% of the total disease burden (Bornman *et al*., 2012). Malaria is estimated to cause between 300 and 500 million clinical cases with about 700,000 to 1.6 million deaths annually and most of these deaths occur in sub-Sahara Africa (Ahorlu and Koram, 2012).

In Nigeria, malaria accounts for 60% of outpatient visits, 30% hospitalization, and is estimated to be responsible for about 11% of overall maternal mortality, 25% of infant mortality, and 30% of under-five mortality. The disease is particularly virulent among the under-five years of age and pregnant women, due to their low levels of immunity (Ebong *et al*., 2012).

Although the burden of malaria is decreasing, parasite resistance to current antimalarial drugs threaten the prospects of malaria elimination in endemic areas (Chinsembu, 2015). In Africa, the use of indigenous plants plays an important role in the traditional methods of malaria treatment by providing good sources for the detection of novel antiplasmodial compounds (Omoregie and Sisodia, 2012). Recent research on the leaves of *J. curcas* has revealed that the aqueous and methanolic extracts following phytochemical detection and by antiplasmodial sensitivity test were highly effective against the malaria parasite. Thus J. curcas could serve as an effective chemotherapy for the treatment of malaria (Imam *et al.,* 2016). Previous studies have shown efficacy of *J. curcas* against most vector-borne diseases (Ohimain *et al.,* 2014). It has been reported that different parts of this biodiesel producing plant including the leaves, the oil, sap, stem bark and root have numerous health benefits including skin injections, antidote for snake bites, leprosy and rheumatism, treatment of tooth ache and muscular pains, pile, fever, jaundice, gonorrhoea, constipation, heart burn and as purgative as well as contraceptive principle (Agbogidi *et al*., 2013). It is also maintained that J. curcas has antimalarial, insecticidal, anti-cancerous and anti-tumour properties (Agbogidi *et al*., 2013). Development of resistance to almost all known antimalarial drugs including the once highly effective ACT-based therapies has necessitated the need for new compounds for malaria management and since plants has always provided promising and effective therapeutic compounds in the past, it becomes necessary to screen and study many plant species for novel and more effective therapy for malaria (Imam *et al.,* 2016), hence this study.

MATERIALS AND METHODS

Collection of Plant Materials

Jatropha curcas sample was collected from Ringim Local Government Area, Jigawa state in July, 2016 during the raining season. The plant was identified by botanists at the herbarium section of the Department of Biological Sciences, Bayero University Kano, where an Accession Number BUKHAN 0060 was assigned to it.

Preparation and Processing of Plant Extracts

The stem barks of *J. curcas* were collected and air dried under shade at room temperature in the Department of Biochemistry, Bayero University Kano, Nigeria for 16 days. The extraction method used was maceration. The samples were ground into coarse powder using motor and pestle and stored in an air tight bottle prior to analysis. After weighing 170g of the ground sample of the stem bark, it was dissolved in 1100 mL of aqueous solution in an air tight bottle.150g of the sample was also weighed and dissolved in 1000 mL ethanol solution in an air tight bottle. Both were shaken and kept for 48 h. The extracts were filtered using a chess cloth and Whatman filter paper No. 1 (24 cm), to obtain filtrates of the respective solvents of aqueous and ethanol. The aqueous extract was concentrated to dryness using a freeze drier while the ethanol extract was concentrated in a rotary evaporator and allowed to dry at ambient temperature (Veeramuthu *et al*., 2006).

Phytochemical Screening and Quantification

Qualitative phytochemical analysis was carried out to identify plant secondary metabolites such as alkaloids, carbohydrates, cardiac glycosides, anthranol glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids and diterpenes using standard procedures using standard procedures described by Tiwari *et al.,* (2011); and Parekh and Chanda (2007). Some of the detected phytochemicals were quantified using standard procedures described by Obadanomi and Ochuko (2001); Sudduraju and Becker (2003); Jia *et al.,* (1999) Oluwaniyi *et al.,* (2007) and Makkar *et al.,* (2007).

Preparation of *Plasmodium falciparum* Culture Medium

The media was prepared by dissolving 10.4g of the powdered material into one litre 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 (Dacie and Lewis, 1968). 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 1600 medium (Trager, 1982).

Preparation of the Test Concentrations

An electronic digital balance 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

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 at37°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 Antimalaria 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 Geimsa'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;

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 and significant differences between multiple variables were analysed using multivariate analysis. Values of $P<0.05$ were considered statistically significant. All analysis were carried out using the Graph Pad InStat statistical package.

RESULTS

Physical Properties of the Plant Extracts

Two extracts were obtained from *J. curcas* stem bark. The aqueous stem bark extract (ASBE) of the plant was reddish-brown in colour, oily and gummy in texture. The ethanol stem bark extract (ESBE) of the plant was a greenish-black in colour and powdery in texture. Percentage yields of 6.00% (ASBE) and 2.55% (ESBE) were obtained as shown in Table 1.

Table 1: Physical Properties of Stem Bark Extracts from *Jatropha curcas*

Property	ASBE	ESBE
Weight of plant sample (g)	150	170
Weight of plant extract (g)	9.00	4.34
Percentage yield (%)	6.00	2.55
Colour of extract	Reddish-brown	Greenish-black
Texture of extract	Gummy	Powdery

(ASBE)=Aqueous stem bark extract and (ESBE)=Ethanol stem bark extract

Qualitative Phytochemical Analysis

Result of phytochemical screening of aqueous extract showed the presence of alkaloids, cardiac glycosides, anthranol glycosides, saponins, phytosterols, phenols, protein and amino acids and diterpenes. However, carbohydrates, flavonoid and tannins were found to be absent in the aqueous stem bark extract of J. curcas. Result of phytochemicals of ethanol extract showed the presence of alkaloids, cardiac glycosides, saponins, phenols, flavonoids and diterpenes. Carbohydrates, anthranol glycosides, phytosterols, protein and amino acids and tannins were absent in the ethanol stem bark extract of a *J. Curcas* (Table 2).

Phytochemical Constituents of Aqueous Stem Bark Extract and Ethanol Stem Bark Extract of *Jatropha curcas*

Phytochemicals	ASBE	ESBE
Alkaloids	+	+
Carbohydrates	-	-
Cardiac glycosides	+	+
Anthranol glycosides	+	-
Saponins	+	+
Phytosterols	+	-
Phenols	+	+
Flavonoids	-	+
Protein and amino acids	+	-
Diterpenes	+	+
Tannins	-	-

Key; + =Present; - =Absent; ASBE=Aqueous stem bark extract and ESBE=Ethanol stem bark extract

Quantitative Phytochemical Analysis

The quantitative estimation of these secondary metabolites showed that ethanol extract has a higher amount of flavonoid, phenols and saponins than the aqueous extract. Saponin was found to have the highest quantity (164.9024 mg DE/g ± 0.5616 extract in aqueous extract and 173.3504 mg DE/g ± 0.2639 extract in ethanol extract), followed by cardiac glycosides (4.4700 g% ± 0.0447), flavonoids (2.2436 mg RE/g ± 0.0227 extract in ethanol extract), alkaloids (2.2000 g% ± 0.5657) and the least quantity was found in phenols (0.0397 mg GAE/g ± 0.0001 extract in aqueous extract and 0.0917 mg GAE/g ± 0.0008 extract in ethanol extract).

***In Vitro* Antimalarial Activity of Extracts**

The results of antimalarial activity of the extracts are shown in Table 3. Both the aqueous and ethanol stem bark extracts have reflected a remarkable activity at most concentrations with few exceptions. The highest antiplasmodial activity was obtained with both the ethanolic and aqueous extract, in which the microscopic examination of stained slides at 10mg/ml showed a percentage elimination of 77% after 72 hours.

The least antiplasmodial activity was observed with the ethanol extract at a concentration of 1.25mg/ml yielding a percentage elimination of 38%. This is shown in the table below. The development stage of the malaria parasite at which both the aqueous and ethanol extracts at 10mg/ml concentration and 24 hours incubation kill the malaria parasite is at the schizont stage by disrupting the Plasmodial cell membrane.

Table 3: *In Vitro* Antimalarial Activity of *J. curcas* Aqueous and Ethanol Extract with Percentage Elimination after 24, 48 and 72hours Incubation

Solvents	Conc. (mg/ml)	Mean no. of parasites before incubation	Average no. of parasites during incubation			Mean no. of parasites after incubation	Percentage elimination at the end of incubation
			24h	48h	72h		
Positive control- (Artemisin Combination Therapy)	10	10	1.0±0.2	1.0±0.2	0.0±0.0	0.67±0.33	93%
Negative control	-	10	12.0±1.9	15.0±3.2	19.3±3.6	15.4±2.1	0%
Ethanol	10	10	1.6±0.5^{c,n}	2.6±0.2^{p,n}	2.6±0.7^{c,c*,n}	2.27±0.33^{n}	77%
	5	10	1.4±0.5^{t,c,n}	3.4±0.9^{t,t*,p,n}	7.2±2.0^{t*,c*,e,n}	4.00±1.70^{n}	60%
	2.5	10	10.4±2.6^{t,c*,e,p}	4.0±1.1^{t*,p,n}	1.0±0.0^{t**,c,n}	5.13±2.77^{n}	49%
	1.25	10	11.6±3.1^{t,c*,p}	4.2±0.9^{t,t*,p,n}	3.0±1.0^{t*,c,c*,n}	6.27±2.69^{n}	38%
Aqueous	10	10	2.2±0.7^{n}	3.0±0.5^{p,n}	1.8±0.4^{c,c*,n}	2.33±0.35^{n}	77%
	5	10	1.6±0.5^{t,n}	5.0±1.0^{t*,p,n}	1.2±0.4^{t,c,c*,e,n}	2.60±1.21^{n}	74%
	2.5	10	3.8±0.7^{e,p,n}	2.2±0.4^{p,n}	2.6±0.6^{c,n}	2.87±0.48^{n}	71%
	1.25	10	5.2±2.8	6.6±1.8^{p}	0.4±0.2^{c*,n}	4.07±1.88^{n}	59%

n = 5 (number of samples); Values are expressed in mean ± S.E.M.; Data analyzed using one way Anova;
t- Compares significance over time interval at the same concentration and same extract at values of P<0.05.
c- Compares significance at different concentrations on the same time perceived and same extract at values of P<0.05.
e- Compares significance of different extracts under the same concentration and same time interval at values of P<0.05.
p- Significantly different from the positive control at values of P<0.05.
n- Significantly different from the negative control at values of P<0.05.

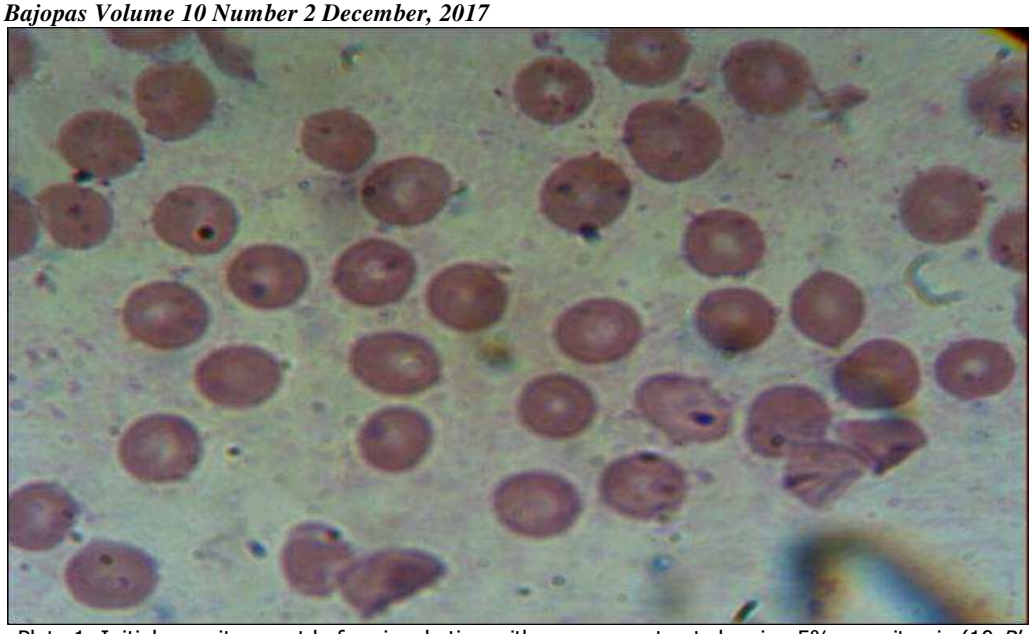

Plate 1: Initial parasite count before incubation with aqueous extract showing 5% parasitemia (10 *Plasmodium falciparum* parasites) using ×100 magnification.

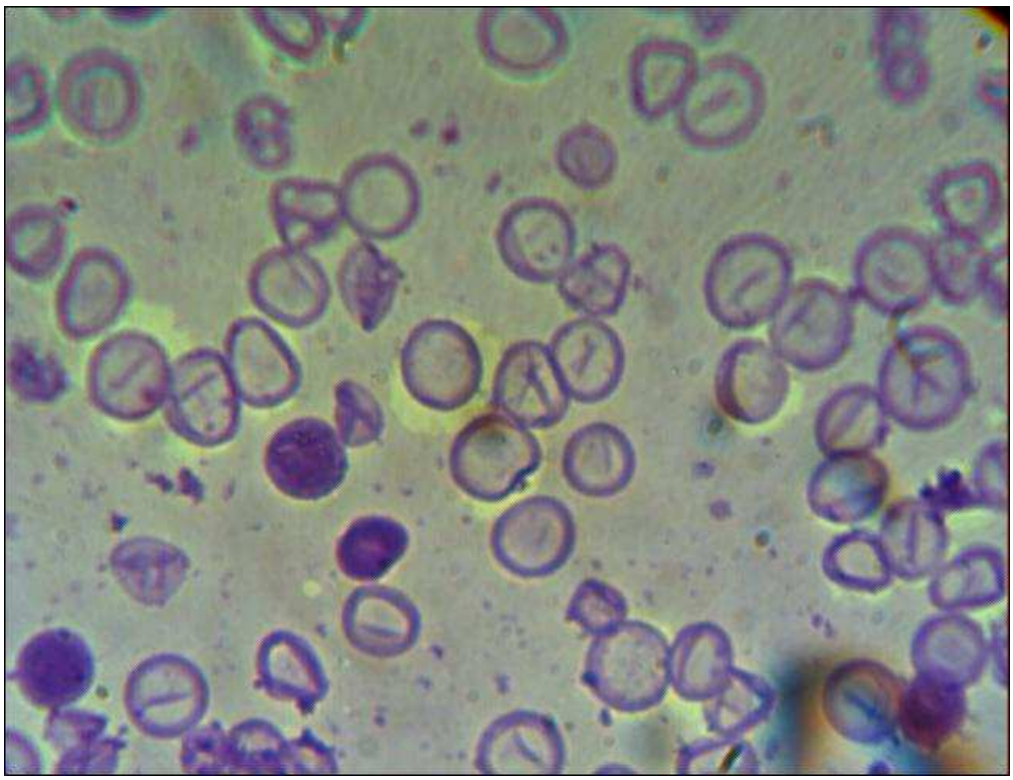

Plate 2: 72hrs after incubation with aqueous extract showing a reduction in parasitemia (No *Plasmodium falciparum* parasites observed) using ×100 magnification.

DISCUSSION

Preliminary qualitative phytochemical screening of the aqueous and ethanol stem bark extracts of *J. curcas* revealed the presence of alkaloids, cardiac glycosides, anthranol glycosides, saponins, phytosterols, phenols, flavonoids, protein and amino acids and diterpenes. Phytochemicals, also called non-nutritive chemicals compounds are secondary metabolites occurring naturally in medicinal plants with various disease defensive properties (Djeridane *et al.,* 2006). The presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids were previously reported in the stem bark of *J. curcas* grown in Southern part of Nigeria (Igbinosa *et al.,* 2009). However, the geographical location of the plant used in the present study was different and tannin was also found to be absent. The phytochemical screening of the aqueous, ethanolic and methanolic extracts of the *J. curcas* has also been reported, but the authors failed to

categorize the bioactive compounds in the three solvents (Igbinosa *et al.,* 2009). Sharma *et al.,* (2012) also reported the presence of bioactive compounds such as alkaloids, saponins, tannins, terpenoids, steroids, glycosides, phenols and flavonoids in the extracts of root, stem and leaf of the plant in methanol extracts.

From table 2, the aqueous extract revealed the presence of more secondary metabolites when compared with the ethanol extract. Both extracts revealed the presence of alkaloids, cardiac glycosides, phenols, saponins and diterpenes whereas carbohydrates and tannins were absent in both extracts. Anthranol glycosides, phytosterols and proteins and amino acids were only found in aqueous extract whereas flavonoid was only found in ethanol extract. These variations could have arisen due to differences in polarity index of the solvents. The variations in these extracts affects the quantity and secondary metabolite composition and thus depends upon type of extraction, time of extraction, temperature, nature of solvent, solvent concentration and most importantly polarity (Ncube *et al.,* 2008). Solvents are either polar or non-polar. Polarity is roughly measured using the dielectric constant of the solvent. Polarity of a solvent is attributed to its attraction power to different compounds in a solution where polar solvents extract polar compounds and non-polar solvents extract non-polar compounds.

Quantitative investigation revealed J. curcas to have saponins in the highest amount. High saponin value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo (Goodrum, 2002).Low saponin levels are considered safe and non-toxic as high saponin levels have been associated with gastroenteritis, manifested by diarrhoea and dysentery (Awe and Sodipo, 2001). Saponins are amphipathic glycosides which are grouped phenomenologically by the soap-like foaming they produce when shakened in aqueous solutions (Hostettmann and Marston, 1995). Most saponins function as antioxidants, because they possess a special moiety (2,3-dihydri-2,5–dihyroxy6-methyl-4-pyran-4-one) which act by forming hydroperoxide intermediates which scavenges free radicals (Hu *et al*., 2002). Saponins also possess haemolytic action on human erythrocytes (Baumann *et al*., 2000). Saponins with acyl residues or oxide-ring moiety tend to show haemolytic activity (Oda et al., 2000).A moderate level of cardiac glycosides was found, which is a class of natural product used to increase the cardiac contractile force in patients with congestive heart failure and cardiac arrhythmias (Hauptman *et al.,* 1999). Flavonoids that have been used for centuries in oriental medicine (Di Carlo *et al.,* 1999) was also present in medium quantity in the plant extracts. Antioxidant properties, reactive oxygen species scavenging, and cell function modulation of flavonoids could be attributed to their pharmacological activity (Limasset *et al*., 1993). The next is alkaloids which are known to precipitate hepatocyte necrosis and cytoskeleton disorganization (Lekhehal *et al.,* 1996). Phenolic alkaloids such as caffeic acid phenyl ester (CAPE) have been reported to possess beneficial effects such as anti-tumour property against human breast cancer line (Grunberger *et al*., 1988) and also in the treatment of acute inflammation (Orban *et al*., 2000).Phenols was found to have the least quantity in the stem bark extract of Jatropha curcas.

The toxicity of Jatropha plant can be attributed to the presence of secondary metabolites. Alkaloids are found to inhibit the cell's central processes, an important requisite property against pathogens (Likhitwitayawuid *et al.,* 1993). Secondary metabolites such as alkaloids have been implicated in antiplasmodial activity in many plants (Okokon *et al.,* 2006). Alkaloids also exhibit toxicity against foreign cells in culture and are widely studied in cancer research. On the other hand, saponins disrupt the cell wall providing toxic materials free access to the cell. The damage to the cell wall leads to leakages of valuable cell constituents that are usually stored inside (Oyi *et al*., 2007). Phenolics and flavonoids act as cytoplasmic poisons with their inhibitory effect on cellular enzymes (Iwu, 1985). Flavonoids has been revealed to possess significant anti-parasitic activities against various strains of malaria parasite, trypanosome and leishmania (Al-Adhroey *et al.,* 2010). Flavonoids also are known for their antimicrobial, cytostatic and anti-inflammatory properties (Hodek *et al.,* 2002). The antimicrobial action of most of the Jatropha plant can also be attributed to the presence of phenolics (Kowalski &Kedzia, 2007; Igbinosa *et al*., 2007). These compounds act as acids with high antimicrobial action (Oyi *et al.,* 2007). Aside from their very potent antibacterial action, phenolics also exhibit anticancer, antitumor and cytotoxic properties (Aiyelaagbe *et al.,* 2007; Igbinosa, 2009).

From Table 3, it can be seen that the average number of parasites after incubation 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. The elimination rate for both the extracts was seen to be almost rapid within the first 24hrs, which then decreased steadily within the next incubation periods. Besides, previous studies on J. curcas leaves showed that it contained some phytochemicals which includes alkaloids, saponins, tannins, flavonoids, phenols, glycosides, steroids and terpenoids. The aqueous and methanolic extracts showed a remarkable antiplasmodial activity with high percentage eliminations of 95.4% (Imam *et al.,* 2016). However in this study at the highest dose of 10mg/ml, both the ethanol and aqueous extracts showed percentage elimination of 77%. The least activity was observed with ethanol extract at 1.25mg/ml which yielded a percentage elimination of 38%. This shows that the leaf of *J. curcas* could be more effective than the stem bark in the treatment of malaria. Also the aqueous extract was observed to have higher percentage elimination at the various concentrations when compared with the ethanol extract. This could be due to the aqueous extract dissolving more phytochemicals than the ethanol extract. These observations suggest that the activity of the extracts may be cytotoxic for the malaria parasite, there by inhibiting their growth and development.

CONCLUSION

This study revealed the presence of various phytochemicals which was ascertained by both qualitative screening and quantitatively screening. The aqueous and ethanol stem bark extracts of *J. curcas* was found to have antiplasmodial properties at higher concentrations of both extracts. This suggests that *J. curcas* contains secondary metabolites with promising antimalarial properties which when fully harnessed could offer a novel therapy for the management of clinical malaria.

Recommendation

The promising antiplasmodial properties of *J. curcas* established in this study could form a basis for recommending herbal preparation of the plant for management of malaria. However, care should be taken in its usage until extensive toxicological studies is conducted to establish its safety or otherwise.

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Conflict of interest

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

Author's contribution

Author AAI conceived the idea, designed the experiment, supervised the lab work and wrote the first draft of the manuscript. Author AS conducted the lab work and performed statistical analysis. Authors IAK and HA supervised the statistical analysis and literature search and review. All authors read and approved the manuscript.

REFERENCES

Agbogidi, O.M., Akparobi, S.O., and Eruotor, P.G. (2013). Health and Environmental Benefits of *Jatropha curcas Linn*. *Applied Science Reports*, 1(2), 36-39.

Ahorlu, K.C., and Koram, K.A. (2012). Intermittent Preventive Treatment for Children (IPTC) Combined with Timely home treatment for malaria control. *Malaria Journal 2012*, 11(Suppl 1):P108.

Aina, V.O., Ibrahim, M.B., Abdulsalam, M.S., Adejo, G.O., and Solomon, M. (2016). Anti diabetic Activities of the Leaf and Bark Extracts of *Jatropha Curcas* on Alloxan-Induced Diabetic Albino Rats. *Journal of Natural Sciences Research*, 6(4), 91-95.

Aiyelaagbe, O.O., B.A. Adeniyi, O.F. Fatunsin and B.D. Arimah, (2007). In vitro antimicrobial activity and phytochemical analysis of Jatropha curcas roots. *Int. J. Pharmacol.*, 3: 106–110.

Al-Adhroey, A.H., Nor, Z.M., Al- Mekhlafi, H.M., Amran, A.A., and Mahmud, R. (2001). Antimalarial Activity of Methanolic leaf extract of *Piper betle*. *L. Molecules*. 16: 107-118

Awe IS, Sodipo O A (2001). Purifications of saponins of root of Blighia sapida. *Niger J Biochem Mol Biol*. 16(3): 201-4.

Baumann E, Stoya G, Volkner A, Richter W, Lemke C, Linss W (2000). Haemolysis of human erythrocyte with saponin affects the membrane structure. *Acta Histochemica* 102(1):21-35.

Bornman, M., Kylin, H., and Bouwman, H. (2012). Household behavioural responses following successful IRS malaria control: challenges for health education and intervention strategies. *Malaria Journal 2012*, 11(Suppl 1):P12.

Chinsembu, K.C. (2015). Plants as antimalarial agent in Sub-Saharan Africa. *Acta Tropica* 152, 32-48.

Dacie, J.V. and Lewis, S.M. (1968). Eds. Practical Haematology, Fourth edition. Blackwell Publishers, USA, pp 65-66.

Di Carlo G, Mascolo N, Izzo A A, Capasso F (1993). Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci.* 64(4):337-353.

Djeridane A., M Yousfi B. Nadjemi D. Boutassouna, P Stocker and N. Vidal (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds food Chem. 97: 654-660.

Ebong, O.O., Asuquo, E.O., Nwauche, C.A., Siminialayi, I.M., Ogbuehi, I.J., and Ajienka, M.F. (2012). Antimalarial treatment by health care providers in Port Harcourt, Nigeria. *Malaria Journal 2012*, 11(Suppl 1):P28.

Goodrum, J. W., 2002. Volatility and boiling points of biodiesel from vegetable oils sand tallow. *Biomass Bioenergy* 22: 205–211.

Grunberger D, Banerjee R, Eisinger K, Oltz EM, Efros L, Caldwell M, Estevez V, Nakanishi K (1988). Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. *Experientia* 44(3):230-232.

Hodek, P., P. Trefil and A. Stiborova, 2002.Flavonoids-potent and versatile biologically active compounds interacting with cytochrome P450. *Chemico-Biological Interactions,* 139: 1–21.

Hostettmann K, Marston A (1995).Saponins Chemistry and Pharmacology of National Products. University Press Cambridge. p560.

Hu J, Lee SO, Hendrich S, Murphy P. A (2002). Quantification of the group B soya saponins by high performance liquid chromatography. *J. Agric. Food Chem*. 50:2587-2594.

Igbinosa, O.O., Igbinosa, E.O., and Aiyegoro, O.A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology*, 3(2), 058-062.

Imam, A.A., Salim, M.A., Bala, M., Aisha, M.I. and Yahaya, S. (2016). Phytochemistry and antiplasmodial properties of aqueous and methanol leaf extracts of *Jatropha*. *Bayero journal of pure and applied sciences,* 9(1):93-98.

Iwu, M.M., 1985. Antihepatotoxic constituents of Garcinia kola seeds. *Experientia,* 41: 699–670.

Jia, Z., Tang, M., and Wu, J. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*, 64(4):555-559.

Kowalski, R. and B. Kedzia, (2007). Antibacterial activity of *Silphium perfollatum* extracts. *Pharm. Biol*., 45: 495–500.

Lasalita-Zapico, F. C., Aguilar, C.H.M., Madas, J.B., and Eroy, M.N. (2012). Chemical composition, antimicrobial properties and toxicity of *Jatropha curcas* provenances from diverse origins*. International Journal of Agriculture and Biology*, 14, 625–628.

Lekhehal M, Pessayre D, Lereau JM, Moulis C, Fouraste F, Fau D (1996). Hepatoxicity of the herbal medicine germander. Metabolic activation of its furanoditerpenoids by cytochrome P450 3A depletes cytoskeleton associated protein thiols and forms plasma membrane bebs in hepatocytes. *Hepatology* 24:212 -221.

Likhitwitayawuid, K., C.K. Angerhofer, H. Chai, J.M. Pezzuto, G.A. Cordell and N. Ruangrungsi, 1993. Cytotoxic and antimalarial alkaloids from the tubers of *Stephaniapierrei.J. Nat. Prod*., 56: 1468–1478.

Limasset B, Doucen CL, Dore JC, Ojasoo T, Damon M, Paulet AC (1993). Effects of flavonoids on the release of reactive oxygen species by stimulated human neutrophils. *Biochem.Pharmacol*. 46:1257–1271.

Makkar HP, Siddhuraju P, and Becker K. (2007. Methods in Molecular Biology; Plant Secondary Metabolites. *Totowa: Human press*; 2007.pp. 93-100.

Mbakwem – Aniebo, C. Okoyomo, E.P., Ogugbue, C.J., and Okonko, I.O. (2012). Effects of *Jatropha curcas* leaves on common Dermatophytes and causative agent of Pityriasis versicolor in Rivers State, Nigeria. *Nature and Science 2012*, 10(12), 151-157.

Mukhtar, M.D., Bashir, M., and Arzai, A.H. (2006). Comparative In vitro Studies on Antiplasmodial activity of some Nigerian and Foreign brands of chloroquine Oral formulations marketed in Kano. African Extract of *Piper betle* L. Molecules, 16, pp 107-118.

Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology* 2008; 7 (12): 1797-1806.

Obadoni, B.O., and Ochuko, P.O. (2001).Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. *Glob J Pure Appl Sci*, 8(2):203-208.

Oda, K., Matsuda H, Murakami T, Katayama S, Ohgitani T, Yoshikawa M (2000). Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. *Biol. Chem.* 381(1):6 –74.

Ohimain, E.I., Tariwari, C.N., and Sunday, E.B. (2014). Comparative larvicidal activities of the leaves, bark, stem and root of *Jatropha curcas* (Euphorbiaceae) against malaria vector Anopheles gambiae. *Sky Journal of Biochemistry Research*, 3(3), 029 – 032.

Okokon, J.E.; Ita, B.N. and Udokpoh, A.E. (2006). Antiplasmodial activity of *Cylicodiscus gabunensis*. *Journal of Ethnopharmacology*, 2, pp 175-178.

Oloyede, O. B., Salau, A. K., Akeusola, R. T., Ganiyu, O. T., Azeez, L., and Ogunbode, S. M. (2012). Phytochemical Content, Radical Scavenging and Antibacterial Properties of Aqueous Extract of *Jatropha curcas Linn* Leaves*. Fountain Journal of Natural and Applied Sciences*, 1(1), 41 – 48.

Oluwaniyi, O.O., Ibiyemi, A.S., Usman, A.L. (2007). Effect of Detoxification on the Nutrient content of Thevetia peruviana seed cake, *Res. J. Applied Sci*., 2(2)188-191.

Oluwaniyi, O.O., Ibiyemi, A.S., Usman, A.L. (2007). Effect of Detoxification on the Nutrient content of Thevetia peruviana seed cake, *Res. J. Applied Sci*., 2(2)188-191.

Omoregie, E.S., and Sisodia, B.S. (2012). In vitro antiplasmodial activity and cytotoxicity of leaf extracts of *Jatropha tanjorensis* J.L. Ellis and Soroja. *Bayero Journal of Pure and Applied Sciences*, 5(1), 90 – 97.

Oyi, A.R., J.A. Onaolapo, A.K. Haruna and C.O Morah, 2007. Antimicrobial screening and stability studies of crude extract of Jatropha curcas Linn latex (Euphorbiaceae). *Nigerian. J. Pharmace.* Sci., 6: 14–20.

Parekh, J. and Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr.J.Biomed.Res*, 10:175-181.

Sharma AK, Gangwar M, Tilak R, Nath G, Sinha ASK, Tripathi YB, Kumar DC (2012).Comparative in vitro antimalarial and phytochemical evaluation of methanolic extract of root, stem and leaf of *Jatropha curcas* Linn. *Intl. J. Pharm. Sci. Res*. 3(6):154-1553.

Siddhuraju, P., and Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *J. Agric Food Chem*, 51(8):2144-2155.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, 1(1)98-106.

Trager, W. (1982). Cultivation of malaria parasites. *British Medical Bulletin.* 38(2), pp 129 – 131.

Veeramuthu, D.; Muniappan, A. and Savarimuthu, I. (2006). Antimicrobial activity of some of some ethnomedicinal plants used by paliya tribe from Tamil Nadu India. Published online, October, 2006. doi,10.1186/1472-6882-6-35.