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Qualitative Phytochemical Screening and Larvicidal Efficacy of Physic Nut (*Jatropha curcas*) Leaves, Stem-bark and Root Extracts on Mosquito Larvae

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The advance of battle by mosquito species to artificial chemicals has obliged the unrelenting pursuit of nontoxic ones from plants. This study screened the qualitative phytochemicals and evaluated the larvicidal efficacy of extracts of *Jatropha curcas* leaves, stem-bark and root extracts against third and fourth mosquito instar larvae. The *J. curcas* obtained were subjected to extraction by maceration. Qualitative phytochemical screening of ethanolic stem-bark, aqueous and ethanolic root extracts plants showed the presence of phenols, saponins, steroids flavonoids, alkaloids, glycosides, carbohydrates and terpenoids. The aqueous extracts of the leaves and stem-bark showed the presence of alkaloids, cardiac glycosides, phenols, saponins, and terpenoids whereas, ethanolic leaves extract had similar phytochemicals with the leaves and stem-bark aqueous extracts but did not have steroids. Twenty-four (24) hours exposure of different concentrations (2.0mg/ml, 4.0mg/ml, 6.0mg/ml, 8.0mg/ml, 10 mg/ml) of various extracts showed that larval

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mortality increased significantly (P<0.05) with increase in extracts concentration. Aqueous stem-bark extract showed highest mortality with 87% against third mosquito instar larvae followed by aqueous, ethanolic root extracts against third and fourth mosquito instar larvae respectively with 85% larval mortality. However, least mortality was observed in ethanolic leaf extract with 5% larval mortality against fourth mosquito instar larvae. Low LC_{50} and LC_{90} values were noticed in ethanolic root and aqueous stem-bark extracts with 2.19mg/ml and 11.51mg/ml respectively. Whereas the highest LC_{50} and LC_{90} values were noticed in both ethanolic leaves extracts with 14.09mg/ml and 26.20mg/ml respectively against fourth mosquito instar larvae. Conclusively, aqueous stem-bark and ethanolic root extracts can be harnessed to control 50% and 90% mosquito instar larval mortality respectively. However, there is a need to ascertain the quantities of bioactive components of *J. curcas* and its toxicity to non-target organisms.

Keywords: Jatropha curcas; larvicidal efficacy; phytochemical; mosquito larvae.

1. INTRODUCTION

Mosquitoes are very important vectors of several human diseases including malaria, lymphatic filariasis, dengue virus and Zika virus worldwide [1]. Mosquito-borne diseases are the major public health problems in developing countries. Malaria parasites are conveyed to humans by female Anopheles mosquitoes [2]. The parasites are propagated in humans through the bites of infected Anopheles mosquitoes. There are more than 400 species of Anopheles mosquitoes have been known and of these, 60 species serve as hosts to *Plasmodium*. *Plasmodium* is a parasitic protozoan that causes malaria. There are five types of human malaria parasites: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium knowlesi Plasmodium knowlesi* naturally infect macaques in Southeast Asia and also infects humans, causing malaria that is transmitted from animal to humans "zoonotic malaria"[2]. Yellow fever is endemic in most part of Nigeria which causes lives and economy loss. Lymphatic filariasis which is transmitted by *Aedes* and *Culex* species, affects more than 120 million people, and about 1.4 billion are at risk of these diseases in 73 countries which is rampant in African and Asian countries. Although, these diseases are associated with health and socioeconomic consequences [3].

Mosquito can be controlled by preventing mosquito bite using mosquito repellants, killing mosquitoes and causing larval mortality [4]. The larval stage is the most vulnerable stage to attack mosquitoes as they are concentrated in smaller areas. Thus one of the approaches for control of malaria transmission is by interrupting the mosquito life cycle at the larval stage. Vector control for the prevention of malaria includes insecticide treated bed nets, indoor residual spraying and source reduction (larval control) [4]. Resistance to these insecticide classes has emerged in anopheles' mosquitoes [5] and its rapid spread has become a major obstacle to vector control [6]. Nigeria has the vision to become a malaria-free country with a mission to free every Nigerian citizen from the menace of malaria through effective leadership, environmental sanitation, health system strengthening and coordination in developing efficient policies, strategies and guidelines [7].

The unpredictable mosquito larvae and pupae population transition dynamics, especially during transmission seasons remain a critical issue in the tropics. Ability to carefully address and smashed this vague indicator has eventually determined the level of success in attaining the malaria elimination agenda. This should be significantly reduced through correct, consistent and effective application of Larval Source Management (LSM), which targets mosquito larvae as they mature in aquatic habitats [8]. However, extract from plants may serve as an alternative source of bioactive compounds that are biodegradable into non-toxic products and are potentially suitable for use to control mosquitoes. Plant extracts in general have best recognized as an important natural resource of insecticides [9]. The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species [9,10].

Phytochemicals derived from plant sources act as larvicides, insect growth regulators, repellents, oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne diseases to the individual as well as at the community level [11]. Therefore, larviciding is one of the successful way of reducing mosquito densities in their breeding

places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides such as organophosphates. Although effective and its repeated use has disrupted natural biological control systems and sometimes result in the wide spread development of resistance [12]. These problems have warranted the need for developing alternative strategies using eco-friendly products. Plants often have an alternative source of insect control because they contain a range of bioactive chemicals many of which are selective and have little or no harmful effect on non-target organisms and the environment. Much effort has, therefore, been focused on plant extracts or phytochemicals as potential sources of mosquito control agents or as lead compounds [9].

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

A portion of *Jatropha curcas* containing leaves and fruits were collected within Sangere-FUTY premises and was transported to the laboratory of the plant department, school of life science, Modibbo Adama University of Technology Yola, for identification.

2.2 Phytochemical Analysis

Ethanolic and aqueous extracts of *Jatropha curcas* leaves, stem-bark and root were qualitatively screened for the presence of tannins, saponins, phenol, carbohydrate, alkaloids, flavonoids, streroids, terpenoids and cardiac glycosides were identified by characteristic color change as described [13,14].

2.3 Collection and Rearing of Mosquito Larvae

Mosquito larvae were collected from the study area (MAUTECH) campus and transported to Biochemistry laboratory (Old School of Pure and Applied Science) of Modibbo Adama University of Technology Yola, for rearing in a plastic bowl containing water covered with nylon mesh. Glucose was used in feeding the mosquito larvae [15].

2.4 Plant Extraction Process

For the crude aqueous extraction, 100 g of the leaves, stem-bark and root of *Jatropha curcas* were pounded with mortar and pestle. The filtrate (juice) was squeezed out using a muslin cloth and concentrated to dryness at room temperature with a rotary evaporator. While for the ethanolic extraction of 100 g of shade-dried leaves, stem-bark and root at room temperature (27±2°C), *Jatropha curcas* were macerated in 90% ethanol for 72 h. The filtrate was then concentrated to dryness using a rotary evaporator and stored in amber bottles in a refrigerator (-4°C) until it was ready for use.

2.5 Preparation of Stock Solution

0.1 gram of ethanolic and aqueous extracts of *Jatropha curcas* leaf, stem-bark and root each were dissolved in 100 millilitre of distilled water to form a stock solution. Serial dilution to obtain different concentrations of 10mg/ml, 8mg/ml, 6mg/ml, 4mg/ml and 2mg/ml were prepared by pipetting 5ml, 4ml, 3ml, 2ml and 1ml respectively of stock solution and they were added to 45ml, 46ml, 47ml, 48ml and 49ml of distilled water containing mosquito instar larvae respectively.

2.6 Larvicidal Bioassay

The third and fourth mosquito larvae, twenty were exposed to different concentrations with slight modifications [15]. Three replicates along with the control were run simultaneously on each trial. The larvae were fed with glucose. The number of dead larvae at 24 hours was recorded. Moribund larvae were also counted as dead larvae. The dead larvae were removed soon after mortality to prevent decomposition, which may cause the rapid death of the remaining larvae. A total of three trials was carried out. The corrected mortality was analysed using Abbot Formulae.

$$\text{Percentage mortality (\%)} = \frac{\text{Number of Dead larvae}}{\text{Number of larvae introduced}} \times 100$$

2.7 Data Analysis

Larval mortality data of the third and fourth mosquitoes' larvae were subjected to Probit analysis to determine the median lethal concentration (LC_{50}) and lethal concentration ninety (LC_{90}) of each extracts against mosquito larvae. Fiducial limits of upper and lower confidence limits of LC_{50} were determined [16]. All data were tested at $P<0.05$ level of significance.

3. RESULTS

3.1 Phytochemical Analysis

The results of ethanolic stem-bark, ethanolic and aqueous root extracts of *Jatropha curcas* showed the presence of alkaloids, cardiac glycosides, saponins, terpenoids, carbohydrate, phenols, flavonoids and steroid but tannins were absent (Table 1). Leaf and stem-bark aqueous showed nearly the same phytochemicals as the above extracts but tannins, flavonoids and carbohydrates were absent. Whereas, ethanolic leaf extracts revealed only alkaloids, cardiac glycosides, phenols, saponins and terpenoids. Tannin, steriods, flavonoids and carbohydrates were absent.

3.2 Toxicity of *Jatropha curcas* Aqueous Extracts on Fourth and Third Mosquito Larvae after Twenty-Four (24) Hours of Exposure

The toxic effect of aqueous leaf extracts of *Jatropha curcas* against the fourth instar was observed at highest concentration (10mg/ml) with 30% mortality, followed by 25%, 20%, 20% and 15% at 8mg/ml, 6mg/ml, 4mg/ml and 2mgml respectively. However, it showed highest larval mortality of 70% against third mosquito instar larvae at 10mg/ml followed by 53%, 33%, 15% and 10% at respective concentrations of 8mg/ml, 6mg/ml, 4mg/ml and 2mg/ml (Table 2).

The toxic effect of aqueous stem-bark extracts of *Jatropha curcas* against fourth instar was observed at highest concentration (10mg/ml) with 85% mortality, followed by 65%, 55%, 45% and 35% at 8mg/ml, 6mg/ml, 4mg/ml and 2mgml respectively. However, it showed highest larval mortality of 87% against third mosquito instar larvae at 10mg/ml followed by 75%, 60%, 40% at respective concentrations of 8mg/ml, 6mg/ml, 4mg/ml and the least mortality was observed at 2mg/ml with 40% mortality (Table 2).

The toxic effect of aqueous root extracts of *Jatropha curcas* against fourth instar was observed high at highest concentration (10mg/ml) with 60% mortality, followed by 40%, 40%, 30% and 25% at 8mg/ml, 6mg/ml, 4mg/ml and 2mgml respectively. However, it showed highest larval mortality of 85% against third mosquito instar larvae at 10mg/ml followed by 60%, 45%, 35% at respective concentrations of 8mg/ml, 6mg/ml, 4mg/ml and the least mortality was observed at 2mg/ml with 25% mortality (Table 2).

Table 1. Phytochemical constituents of leaves, stem-bark and root extracts of *J. curcas*

Phytochemicals	ELE	ALE	ESBE	ASBE	ERE	ARE
Alkaloids	+	+	+	+	+	+
Carbohydrates	-	-	+	-	+	+
Cardiac glycosides	+	+	+	+	+	+
Flavonoids	-	-	+	-	+	+
Phenols	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Steriods	-	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Tannins	-	-	-	-	-	-

Key; + = Present; - = Absent; ELE = Ethanol leaves extract; ALE = Aqueous leaves extract; ESBE = Ethanol stem-bark extract; ASBE = Aqueous stem-bark extract; ERE = Ethanol root extract; ARE = Aqueous root extract

Table 2. Mean mortality of mosquito larvae exposed to different doses of *Jatropha curcas* aqueous extracts

Larvae	Concentrations (mg/ml)	Aqueous leaves extract	Aqueous stem-bark extract	Aqueous root extract
Third	2	2 (10%)	8 (40%)	5(25%)
	4	3 (15%)	8 (40%)	7(35%)
	6	7 (35%)	12(60%)	9(45%)
	8	11(55%)	15(75%)	12(60%)
	10	14(70%)	18(87%)	17(85%)
Fourth	2	3(15%)	7(35%)	5(25%)
	4	4(20%)	9(45%)	6(30%)
	6	4(20%)	11(55%)	8(40%)
	8	5(25%)	13(65%)	8(40%)
	10	6(30%)	17(85%)	12(60%)

3.3 Toxicity of *Jatropha curcas* Ethanolic Extracts on Fourth and Third Mosquito Larvae after Twenty (24) Hours of Exposure

The toxic effect of ethanolic leaves extract of *Jatropha curcas* against fourth instar was observed at highest concentration (10mg/ml) with 45% mortality, followed by 40%, 25%, 15% and 5% at 8mg/ml, 6mg/ml, 4mg/ml and 2mgml respectively. However, it showed highest larval mortality of 60% against third mosquito instar larvae at 10mg/ml and 8mg/ml followed by 55%, 45% and 35% at respective concentrations 6mg/ml, 4mg/ml and 2mg/ml (Table 3).

The toxic effect of ethanolic stem-bark extracts of *Jatropha curcas* against fourth instar was observed at highest concentration (10mg/ml) with 80% mortality, followed by 70%, 65%, 50% and 40% at 8mg/ml, 6mg/ml, 4mg/ml and 2mgml respectively. However, it showed highest larval mortality of 65% against third mosquito instar larvae at 10mg/ml followed by 55%, 50%, 50% at respective concentrations of 8mg/ml, 6mg/ml, 4mg/ml and the least mortality was observed at 2mg/ml with 45% mortality (Table 3).

The toxic effect of ethanolic root extracts of *Jatropha curcas* against both third and fourth mosquito larvae was the same. At highest concentration (10mg/ml) 85% larval mortality was observed, followed by 75%, 65%, 60% at 8mg/ml, 6mg/ml, 4mg/ml respectively and the least mortality was observed at 2mg/ml with 50% mortality (Table 3).

3.4 LC_{50} and LC_{90} of *Jatropha curcas* Plant against Third and Fourth Mosquito Instar Larvae

The highest and lowest LC_{50} value of ethanolic leaf extract was 10.054mg/ml and 7.702mg/ml respectively; the highest and lowest LC_{50} of aqueous leaf extract of *J. curcas* were 14.090mg/ml and 7.744mg/ml respectively. Whereas, the lowest and highest LC_{50} values of ethanolic stem-bark is 3.794mg/ml both (Table 4). But, the aqueous stem-bark showed 3.794mg/ml and 4.672mg/ml as the lowest and highest values. However, the lowest and highest value of aqueous root extract were 6.357mg/ml and 2.189mg/ml respectively while the highest and lowest LC_{50} of ethanolic root extract of *J. curcas* were 8.317mg/ml and 5.831mg/ml and. Highest and lowest values of aqueous extract were 6.357mg/ml and 2.289 respectively (Table 4). While highest and lowest LC_{90} of *Jatropha curcas* ethanolic leaf extract were 17.183mg/ml and 13.011mg/ml; the highest and lowest LC_{90} of aqueous leaf extract of were 126.196mg/ml and 13.291mg/ml respectively. Whereas, the highest and lowest LC_{90} value of ethanolic stem-bark 13.218mg/ml both. However, the lowest and highest LC_{90} value of aqueous root extract were 12.648mg/ml and 15.260mg/ml while highest and lowest LC_{90} of ethanolic root extract of *Jatropha curcas* were 16.441mg/ml and 12.648mg/ml respectively.

4. DISCUSSION

The result of this study reveals the efficacy of aqueous and ethanolic extracts of root, stem bark and leaf of *Jatropha curcas* against third and fourth instar larvae. The ethanolic extract of all the plant parts recorded the highest larvicidal activity on both third and fourth mosquito instar larvae than the aqueous extracts. Although ethanol is polar, but it also has the ability to attract non-polar molecules due to ethyl group of ethanol being non-polar. This contributes to its ability to extract highly polar and non-polar components from the plant material. It has very low toxicity, completely miscible in water, volatile and easily removed from plant material at low temperature [17].

The phytochemical screening of ethanolic stem-bark, ethanolic root and aqueous root extracts, recorded the presence of carbohydrates, cardiac glycosides, phenols, terpenoids, flavonoids, steriods, saponins and alkaloids. This is in agreement with the research findings, which suggested that the bioactive compounds are responsible for antiplasmodial properties [18,19]. In addition to the absence of tannins in all the extracts, aqueous and ethanolic leaf extracts lack carbohydrates and flavonoids and steroids were additionally absent in ethanolic leaf extract which is following the findings carried by Nwokocha, et al. [20], also in consonance to research findings of Zainab, (2016), who found those bioactive compounds. These bioactive compounds found in the extracts were responsible for antibacterial activities (Zainab, 2016). These discrepancies in bioactive compounds could be as a result of the method of plant material extraction, the solvent used and the geographical location of the plant. Since the soil where plants are established have have a different metallic and non-metallic compound [21]. The aqueous stem-bark extracts revealed the presence of alkaloid, cardiac

Table 3. Mean mortality of mosquito larvae exposed to different doses of *Jatropha curcas* ethanolic extracts

Larvae	Concentrations (mg/ml)	Ethanolic Leaves extract	Ethanolic Stem-bark extract	Ethanolic root extract
Third	2	7 (35%)	9(45%)	10(50%)
	4	9 (45%)	10(50%)	12(60%)
	6	11(55%)	10(50%)	13(65%)
	8	12 (60%)	11(55%)	15(75%)
	10	12 (60%)	13(65%)	17(85%)
Fourth	2	1(5%)	8(40%)	10(50%)
	4	3(15%)	10(50%)	12(60%)
	6	5(25%)	13(65%)	13(65%)
	8	8(40%)	14(70%)	15(75%)
	10	9(45%)	16(80%)	17(85%)

Table 4. Probit Analysis of *Jatropha curcas* extracts against third and fourth mosquito instar larvae

Parts of plants solvent	Instar larvae		LC_{50} (mg/ml)	LC_{90} (mg/ml)	Probit Regression equation
Leaves	Aqueous	L3	7.744	13.291	y=-1.789+0.231x
		L4	14.090	26.196	y=-1.492+0.106x
	Ethanol	L3	7.702	13.011	y=-1.859+0.241x
		L4	10.054	17.183	y=-1.807+0.180x
Stem-bark	Aqueous	L3	3.981	11.507	y=-0.678+0.170x
		L4	4.672	12.141	y=-0.802+0.173x
	Ethanol	L3	3.794	13.218	y=-0.266+0.057x
		L4	3.794	13.218	y=-0.516+0.136x
Root	Ethanol	L3	5.831	11.767	y=-0.516+0.136x
		L4	8.317	16.441	y=-1.312+0.158x
	Aqueous	L3	2.189	12.648	y=-0.268+0.123x
		L4	6.357	15.260	y=-0.915+0.144x

Key: L4 = Fourth mosquito instar larvae; L3 = Third mosquito instar larvae; LC_{50} = Lethal Concentration fifty; LC_{90} = Lethal Concentration ninety

glycoside, steriods, saponins, terpenoids and phenols. In addition, carbohydrates, flavonoids and tannins were found absent. This could be as a result of plant sample's proximity to the soil. Ethanolic and aqueous root extracts recorded 85% larval mortality both on third mosquito instar larvae and 87% larval mortality was shown by aqueous stem-bark extract against fourth mosquito instar larvae. The ethanolic leaf extract is responsible for antimalarial and anti-insecticidal [22]. This is contrary to the report that, says only ethanolic root extract elicits more larval mortality than leaves and stem-bark extract due to phytochemical variations [23].

After exposing the mosquito instar larvae for twenty-four hours, the mean mortality of aqueous extracts was insignificant against third and fourth mosquito instar larvae. There was an increase in mortality of the larvae across the concentration gradient, that is, an increase in concentration elicits an increase in larval mortality. This observation supports the result of Rahuman et al. [24] who reported significant larvicidal activities of *Jatropha curcas* against different mosquito species. The larvicidal activities may be due to the presence of bioactive compounds which are toxic to mosquitoes.

The LC_{50} lowest value of 2.19mg/ml was noticed in ethanolic root extract whose LC_{90} value is 11.77mg/ml. This suggests the potency of the *Jatropha curcas* part used against mosquito larvae. Whereas, the lowest LC_{90} value of 11.51mg/ml was noticed in aqueous stem-bark extract whose LC_{50} value is 3.98mg/ml against third instar larvae This is in consonance to the findings that recorded the lowest LC_{50} value of 3.715mg/ml in the subspecies of Jatropha [25]. A similar study was carried by Gawande et al. [26], who observed LC_{50} value of *Jatropha curcas* aqueous leaf extract at 20.41mg/ml whereas the LC_{90} value was 69.01mg/ml. This could be as result of the strain of the insect pest and part of the plant used. Although the direct comparison may be impossible because of the differences in

the bioassay method used. The result of this study is in consonance with the findings of Manjara et al. [27], who reported that the acetone leaf extracts of *Clausena dentata* are very toxic to *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* fourth instar larvae of mosquito. He recorded an LC_{50} value of 0.15mg/ml and an LC_{90} value of 7.20mg/ml against culex larvae. The LC_{50} value of 0.17mg/ml and LC_{90} value of 1.10mg/ml against Aedes larvae; LC_{50} value of 0.05mg/ml and LC_{90} value of 1.05mg/ml against *Anopheles stephensi* larvae. This disagrees with the findings of Alzeir et al. [28], who noticed LC_{50} value, 12.11µg/ml and LC_{90} value of 30.21µg/ml of aqueous leaf extract of *Annona muricata* to be effective on *Aedes aegypti* larvae; and LC_{50} value of 3.41µg/ml and LC_{90} value of 6.17µg/ml to be effective on *Aedes albopictus* larvae. However, Johnson et al. (2018), who reported that, the chloroform extract of *Dichanthium foveolatum* LC_{50} value is 277.03mg/ml whereas the LC_{50} value of *Leptochloa uniflora*, *Molinera trichocarp*, *Pancratium triflorum* was recorded as 300.56mg/ml, 306.60mg/ml, 318.42mg/ml respectively against culex species larvae. This could be as a result of different parameters used. Thus, the plants extracts that have a promised potency against test organisms.

5. CONCLUSION

In conclusion, presence of the following bioactive compounds such as alkaloids, flavonoids, saponins, steroid, phenol, carbohydrates, terpenoids, cardiac glycosides in solvent extracts *Jatropha curcas* leaves, stem-bark and root were responsible for its larvicidal activities against both the third and fourth mosquito instar larvae. Stem-bark extract was more potent against the third and fourth mosquito instar larvae. However, ethanolic leaves extract of *Jatropha curcas* was less potent against both the third and fourth mosquito instar larvae.

6. RECOMMENDATIONS

i. Further work is needed to identify the potent active component in the extracts responsible for observed larvicidal effects.
ii. There is also the need to investigate the effects of the solvent extracts on other life stages (eggs, pupae and adults) and species of mosquito.
iii. There is a need to include plant extracts for mosquito control operations after safety tests.
iv. There is need to investigate the effects of these extracts on mosquito co-inhabitant non-target aquatic organisms to determine specificity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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