Larvicidal effects of *Jatropha curcas* L. against *Anopheles arabiensis* (Diptera: Culicidea)

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ABSTRACT

Crude and column chromatographic fractions of methanol leaf extract of *Jatropha curcas* were tested for their larvicidal activities against laboratory reared late third instar larvae of *Anopheles arabiensis*. Crude methanol leaf extract of *J .curcas* had similar larvicidal activity to 0.5 ppm Temephos (positive control) at test concentrations ranging from 125 -1000 ppm while column chromatographic fractions (F_1 and F_2) of the crude methanol leaf extract of *J.curcas* showed similar larvicidal activities to 0.5 ppm Temephos at 62.5 and 125 ppm test concentrations. Column chromatographic fraction three (F_3) showed similar larvicidal activity to 0.5 ppm Temephos at 125 ppm test concentration. The LC_{50} and LC_{90} values of crude methanol leaf extract of *J.curcas* were found to be 92.09 and 241.09 ppm, respectively. Toxic activities of column chromatographic fraction one (F_1) (LC_{50}=28.65 ppm; LC_{90} = 49.20 ppm) were nearly equal to that of column chromatographic fraction two [F_2] (LC_{50}= 30.40 ppm; LC_{90} = 49.80 ppm). Least toxicity on the test larvae was observed by column chromatographic fraction three [F_3] (LC_{50} = 80.70 ppm; LC_{90} = 123.70 ppm). Thus, the larvicidal activity of crude methanol leaf extract was not due to the synergistic effects of its fractions. Further studies are recommended to identify larvicidal active ingredients from the active column chromatographic fractions of crude methanol leaf extract of *J. curcas*.

Key words: Malaria vector control, *Anopheles arabiensis,* Botanical larvicides *J. curcas*

1. INTRODUCTION

Different strategies have been devised to reduce the prevalence of malaria globally. The vector control arm of malaria prevalence reduction, using indoor residual spraying (IRS) of houses with synthetic insecticides, is challenged by the appearance of insecticide resistant vectors (Jeffery, 1984). Furthermore, the success of indoor residual spraying of houses to control adult anopheline vectors of malaria depend on mosquitoes resting indoors before or after feeding, the presence of walls and surfaces to be sprayed in human shelters, access to the interior of all houses, willingness people to accept spraying and availability of permanent homesteads (WHO, 2006).

On the other hand, control measures directed against the larval and other immature stages of

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mosquito vectors are useful components of malaria control programs in areas where mosquito breeding sites are accessible and relatively limited in number and size. Unlike the highly mobile flying adult vectors that can easily detect and avoid synthetic indoor residual spray chemicals, immature stages of mosquitoes including larvae are confined within relatively small aquatic habitats and cannot readily escape control measures (Killeen *et al*., 2002). Larval anopheline mosquitoes can be controlled by using synthetic larvicidal chemicals such as temephos, fenthion, malathion, chloropyrifos, and methoprene (ICMR, 2002). However, these life stages of anopheline mosquitoes are reported to develop different level of physiological resistance against the aforementioned synthetic larvicides (Vatandoost and Hanafi-Bojd, 2005). Moreover, mismanaged extensive use of synthetic larvicidal chemicals are reported to result in environmental hazards (ICMR, 2003).

Hence, there is a need to identify target specific, cheap, easy to use and environmentally friendly alternative insecticidal substances from plant materials.

Ethnobotanical and laboratory based studies have revealed the existence of insecticidal plants belonging to different families in different parts of the world. Crude solvent extracts of plant parts belonging to different families, essential oils or their chromatographic fractions are shown to have various levels of bio activity against different developmental stages of malaria vector mosquitoes (ICMR, 2003).

Jatropha curcas L. (Physic nut) is currently used primarily for the production of biodiesel. Besides, it is also used as a medicinal plant. Most parts of the plant are used for the treatment of various human and veterinary ailments. The white latex serves as a disinfectant in mouth infections in children. Its latex contains alkaloids including Jatrophine, Jatropham and curcain with anti-cancerous properties. It is also used externally against skin diseases, piles and sores among the domestic livestock. The leaves of *J. curcas* also contain apigenin, vitexin and isovitexin which along with other factors enable them to be used against malaria, rheumatic and muscular pains (Thomas et al., 2008). Antibiotic activity of *Jatropha* has been observed against organisms including *Staphylococcus aureus* and *Escherichia coli* (Matsuse et al., 1998). Crude stem bark extracts of *J. curcas* were reported to inhibit the growth of pathogenic bacteria and fungi (Igbinosa et al., 2009).

In an attempt to establish the role of *J. curcas* in schistosomiasis control, toxic activities of the seed extracts of the plant were studied against intermediate snail hosts and cercariae of

schistosomes (Rug and Ruppel, 2000). Petroleum ether crude leaf extract of *J. curcas* was shown to have larvicidal effects on vector mosquitoes including *C. quinquefasciatus*, *An. stephensi* and *A. aegypti* (Rahuman et al., 2007; Sakthivadivel and Daniel, 2008).

In Ethiopia, about 33 medicinal plants belonging to 27 families have been screened for their mosquito larvicidal activities (Debella et al., 2007). However, compared to the diversity of plant species in general and medicinal plants in particular (Bekele, 2007), and the abundance of nuisance as well as malaria vector mosquitoes (WHO, 2007), studies on plant larvicides to control malaria vector mosquitoes are very much limited in this country. To date there is no report on the larvicidal effects of *J. curcas* leaf extracts and their column chromatographic fractions against *An. arabiensis* in Ethiopia and other parts of Africa. Thus, this study aimed at evaluating the larvicidal effects of crude and column chromatographic fractions of methanol leaf extract of *J.curcas* against laboratory reared late third instar larvae of *An. arabiensis*.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

Leaves of *Jatropha curcas* L. were collected from Lante Town, 486 km south of Addis Ababa, on the road to Arbaminch, in November 2008. Besides the leaves, other representative samples of the plant including fruits and seeds were also collected and species identification was done at the National Herbarium of the Department of Biology, Addis Ababa University. Voucher specimens were deposited in the same Herbarium.

2.2. Processing of the Plant Material

Leaves of *J. curcas* were air dried under shade and ground using a grinding mill (Straub Model 4-E. Philadelphia USA). The powdered leaf material was macerated with methanol in 1:10 (W/V) using Erlenmeyer flasks and placed on orbital shaker (Gallenkamp 5A-4131. England) at room temperature for 72 hours. The leaf extract of *J. curcas* was then filtered through cotton and subsequently with Whatman filter paper (12.5 cm size). Rotary evaporator (Buchi RE 121. Switzerland) was used to remove methanol from the extract. The crude extract was collected in small vials and stored in deep freeze until used in mosquito larvicidal tests.

2.3. Column chromatographic fractionation of the crude leaf extract

Part of the crude methanol leaf extract of *J. curcas* was further subjected to bioassay guided fractionation. Crude methanol leaf extract was fractionated by means of column chromatography using silica gel 60 (0.063-0.2mm mesh size). Column chromatographic elution of crude methanol extract was made using solvent systems including 100% n-hexane (E_1), 50% n-hexane/ 50% chloroform (E_2), 100% chloroform (E_3), 75% chloroform / 25% methanol (E_4) , 50% chloroform / 50% methanol (E_5), 25% chloroform / 75% methanol (E_6) and 100% methanol (E_7). Column chromatographic elluents of crude methanol leaf extract of *J. curcas* were collected in separate flasks and examined by thin layer chromatography (TLC). This was done on silica gel plates (Merck, 60 F254) using methanol/chloroform in 1:4 ratio as a mobile phase. Visualization and identification of spots that indicate constituents of each elluent was done using an Ultra Violet lamp at a wave length of 254 nm. Finally, elluents having similar constituents were pooled and concentrated using rotary evaporator (Innocent et al., 2008; Matasyoh et al., 2008). Column chromatographic elluents, E_1, E_2, E_3 and E_4, of crude methanol leaf extract of *J. curcas* were combined and designated as F_1. Elluent five (E_5) was designated as F_2. Finally, column chromatographic elluents, E_6 and E_7, were combined and designated as F_3. Column chromatographic fractions were collected in separate vials and stored in deep freeze until used in mosquito larvicidal tests.

2.4. Mosquito Larvae

Anopheles arabiensis were obtained from the insectary at the Malaria and other Parasitic and Vector-borne Diseases Research Team, at the Ethiopian Health and Nutrition Research Institute (EHNRI). Mosquitoes were reared using standard procedures. They were kept at 27-28^{o}C. Late third instar larvae were used for all the tests.

2.5. Preparation of Test and Control Solutions

Crude methanol leaf extract of *J. curcas* and its column chromatographic fraction one, F_1, were not readily soluble in water. Therefore, 250 mg of the crude extract as well as its first fraction, F_1, were placed in separate standard measuring flasks and dissolved in 1 ml of acetone. To each of these, mixtures 0.05 ml of Tween 80 was added as an emulsifier. Then, the mixtures were diluted to 250 ml using distilled water to prepare stock solutions of concentration 1000 ppm.

From each of the 250 ml of 1000 ppm stock solution of crude methanol leaf extract and its first fraction, F_1, 125 ml were placed in separate beakers and used as 1000 ppm test concentrations. The remaining stock solutions of crude methanol leaf extract of *J. curcas* and its first fraction, F_1, were diluted with water up to 250 ml and were considered as 500 ppm test concentrations. These sequential methods were used to prepare serially diluted test concentrations including 250, 125, 62.5 and 31.25, 15.63 and 7.83 ppm (Sakthivadivel and Daniel, 2008). A mixture of 1 ml of acetone and 0.05 ml of Tween 80 was diluted to 250 ml in standard measuring flask by adding distilled water to serve as a negative control solution for larvicidal bioassays involving test concentrations prepared from crude methanol leaf extract of *J. curcas* and its first column chromatographic fraction, F_1, (WHO, 2005; Sakthivadivel and Daniel, 2008). Column chromatographic fractions, F_2 and F_3 of crude methanol leaf extract of *J. curcas* were soluble in water. Thus, 250 mg of F_2 and F_3 were placed in separate standard measuring flasks containing 250 ml of distilled water to prepare stock solutions of concentration 1000 ppm. And serially diluted to test concentrations including 500, 250, 125, 62.5, 31.25, 15.63 and 7.83 ppm using similar procedure mentioned above. Equal volume of distilled water was used as a negative control for larvicidal bioassay involving test concentrations prepared from F_2 and F_3. Temephos (0.5 ppm) was used as positive control for larvicidal bioassays involving both crude as well as column chromatographic fractions of methanol leaf extract of *J.curcas.*

2.6. Larvicidal Bioassays

Larvicidal bioassays were conducted for 24 hours in glass beakers of 125 ml test solutions with 12 replicates of each test concentration for crude methanol leaf extracts of *J.curcas* and with 4 replicates of each test concentration for column chromatographic fractions of the crude leaf extract. Batches of 20 late third instar larvae of *An. arabiensis* were transferred into each test concentration of crude methanol leaf extract and column chromatographic fractions by means of droppers. Larval mortalities were recorded after 24 hours of exposure in each concentration of the test solutions. Larvae were confirmed dead when they failed to move after probing them with a needle at their cervical region. Moribund larvae were those incapable of rising to the surface within a reasonable period of time when the test solutions were disturbed. Moribund larvae were counted and added to dead larvae (WHO, 2005).

3. DATA ANALYSIS

Mean percent mortalities of the late third instar larvae of *An. arabiensis* that were treated with crude methanol leaf extract of *J. curcas* and its column chromatographic fractions, F_1, F_2 and F_3, were determined by one-way analysis of variance (ANOVA) using SPSS for windows, version 15 after 24 hours of exposure. When significant difference was observed, the means were separated using Tukey's HSD test. ANOVA was also used to test variation in larval mortalities among F_1, F_2 and F_3 at 125 and 62.5 ppm test concentrations.

For crude as well as column chromatographic fractions of methanol leaf extract of *J. curcas* the LC_{50} and the LC_{90} values were determined using dosage mortality probit regression analyses of SPSS program version 15 to determine their larvicidal efficacies (WHO, 2005).

4. RESULTS

4.1. Larvicidal Activity of Methanol Leaf Extract of *J. Curcas*

The mean % larval mortalities of crude methanol leaf extract and their column chromatographic fractions are given in Table 1 and 2, respectively. All the concentrations tested have incurred mortalities proportional to the level of concentration. The highest mortality for the crude methanol extract was recorded at 500 ppm, 99.56 %, while it was at 125 ppm for the fractions *F1* and *F2*, 100%. Temephos, a standard insecticide, achieved 100% mortality at much lower concentration, 0.5ppm. At the 125 ppm, larval mortalities among F_1, F_2 and F_3 were not significantly different ($P > 0.05$) while at 62.5 ppm, larval mortalities among F_1, F_2 and F_3 were significantly different ($P < 0.01$) (Table 2).

Table 1. Mean % larval mortalities induced by crude methanol leaf extract of *J.curcas* on the late third instar larvae of *An. arabiensis* after 24 hours of exposure.

Treatments	***Concentration (ppm)***	***Mean % mortality ± (SE)***[+]
Temephos	0.5	100(0.00)[a]
Jatropha curcas	1000	99.56(0.44)[a]
	500	99.56(0.44)[a]
	250	94.08(3.96)[a]
	125	71.27(12.54)[a]
	62.5	38.99(12.86)[b]
	31.25	37.23(12.13)[b]
Control	0.00	2.92(1.68)[c]

[+]same letters at each test concentration are not significantly different ($P > 0.05$).

Table 2. Mean % larval mortalities induced by column chromatographic fractions (F_1, F_2 and F_3) of the crude methanol leaf extract of *J.curcas* on the late third instar larvae of *An. arabiensis* after 24 hours of exposure

	Mean % mortality ± (SE) [+]			
	Treatments			
Concentration (ppm)	***Temephos***	***F_1***	***F_2***	***F_3***
0.5	100 ± 0.00^{a}	-	-	-
125	-	$100\ (0.00)^{a}$	$100(0.00)^{a}$	$86.25(8.51)^{a}$
62.5	-	$95.00(5.00)^{ab}$	$96.25(3.75)^{a}$	$42.50(14.50)^{b}$
31.5	-	$64.86(10.47)^{b}$	$55.00(18.82)^{ab}$	$3.75(2.39)^{bc}$
15.63	-	$27.78(12.6)^{bc}$	$26.25(17.72)^{bc}$	$1.25(1.25)^{bc}$
7.82	-	$1.25(1.25)^{c}$	$1.25(1.25)^{bc}$	$1.25(1.25)^{bc}$
0.00	-	$2.50(2.50)^{c}$	$0.00(0.00)^{c}$	$0.00(0.00)^{c}$

[+]same letters at each test concentration of each fraction are not significantly different ($P > 0.05$).

In the larvicidal bioassays involving both the crude and column chromatographic fractions of methanol leaf extract of *Jatroha curcas*, there were no pupation and mortalities that exceeded 20% in the negative control solutions. Thus, discarding the bioassay experiments or correcting the mortality data was not necessary.

Table 3. Lethal concentration 50 and 90 values of crude and column chromatographic fractions of the leaf extract of *Jatropha curcas* on the late instar larvae of *An. arabiensis* after 24 hours of exposure.

Treatment	LC_{50}♦ (95% CI)	LC_{90}◘ (95%CI)	*P-value*
Crude methanol leaf extract	92.09 (…)*	241.09 (…)*	< 0.0001
F_1	28.65 (20.81-40.82)	49.19 (38.04-77.95)	0.002
F_2	30.40 (23.55-40.84)	49.79 (39.72-73.12)	0.009
F_3	80.67 (66.43-100.21)	123.70 (103.38-161.48)	0.026

♦, Lethal concentration to kill 50% of the treated larvae;
◘, lethal concentration to kill 90% of the treated larvae;
*, upper and lower confidence limits were not given by the software for the LC_{50} and LC_{90} values of crude methanol leaf extract because variation in the mortality responses at different test concentration were very low;
CI , confidence interval.

4.2. Determination of LC_{50} and LC_{90} values

The LC_{50} and LC_{90} values for the crude extract and the fractions are summarized in table 3. Crude methanol leaf extract of *Jatropha curcas* had less toxicity against the test larvae (LC_{50} = 92.09 ppm; LC_{90} = 241.09 ppm) as compared to its column chromatographic fractions [F_1] (LC_{50}=28.65 ppm; LC_{90} = 49.20 ppm), [F_2] (LC_{50}= 30.40 ppm; LC_{90} = 49.80 ppm) and [F_3] (LC_{50} = 80.70 ppm; LC_{90} = 123.70 ppm). Toxic activities of (F_1) (LC_{50}=28. 65 ppm; LC_{90} = 49. 20 ppm) were nearly equal to that of column chromatographic fraction two [F_2] (LC_{50}= 30.40 ppm; LC_{90} = 49.80 ppm). Least toxicity on the test larvae was observed by column chromatographic fraction three [F_3] (LC_{50} = 80.70 ppm; LC_{90} = 123.70 ppm) (Table 3).

5. DISCUSSION

There were no previous studies that compared larval mortalities induced by different concentrations of crude and column chromatographic fractions of methanol leaf extract of *J.curcas* to the standard larvicide, (0.5 ppm Temephos), on the late instar larvae of *An. arabiensis*. However, Cetin and Yanikoglu, (2006) compared larval mortalities induced by essential oils of plants, *Origanum onites* and *Origanum minutiflorum*, to larval mortalities induced by 1 ppm Temephos on third and fourth instar larvae of *Culex pipens*. According to their study (Cetin and Yanikoglu,2006), 150 and 200 ppm test concentrations of essential oils from both plants showed similar larvicidal activities to the 1 ppm Temephos ($P > 0.005$). In the present study, test concentrations ranging from 125 to 1000 ppm of crude methanol leaf extract of *J. curcas* had same effect as 0.5 ppm Temephos ($P > 0.05$).

The present study revealed crude methanol leaf extract of *J. curcas* and its column chromatographic fractions to have larvicidal activities with the LC_{50} values of < 100 ppm on the late third instar larvae of *An. arabiensis*. Although a different solvent system was used, this is consistent with the study results of Sakthivadivel and Daniel (2008), who showed crude petroleum ether leaf extract of *J. curcas* to have larvicidal activity with the LC_{50} of < 100 ppm on the early fourth instar larvae of vector mosquitoes including *C. quinquefasciatus*, *An. stephensi* and *A. aegypti*. Nevertheless, the reasons for the high discrepancy shown between the studies of Sakthivadivel and Daniel (2008); and Rahuman et al. (2007) using the same solvent system is not clear. In the latter study (Rahuman et al., 2007) , crude petroleum ether leaf extract of *J. curcas* was shown to have high larvicidal potency on the early fourth instar larvae of *A.*

aegypti and *C. quinquefasciatus* with the LC_{50} values of 8.79 and 11.34 ppm, respectively (Rahuman et al., 2007).

For column chromatographic fractions, F_1, F_2 *and* F_3, the upper higher concentrations including 1000, 500 and 250 ppm were discarded and larvicidal tests were conducted using lower test solutions of concentration ranging from 125 -7.82 ppm. This was due to the fact that column chromatographic fractions are expected to contain larvicidal active ingredients.

No previous study was reported on the larvicidal activities of column chromatographic fractions of *J. curcas* leaf extracts on *An. arabiensis* or other malaria vector mosquitoes. In the present study, column chromatographic *fractions* of crude methanol leaf extract of *J. curcas* showed enhanced larvicidal efficacy, suggesting that the larvicidal activity of crude methanol leaf extract of *J. curcas* was not due to the synergistic effects of its fractions. Enhanced larvicidal activity of column chromatographic fractions of leaf extracts were also reported earlier by Innocent et al. (2008) showing column chromatographic fractionation of the crude dichloromethane extract of the root bark of *Lantana viburnoides* to result in improved larvicidal activity against the late third and fourth instar larvae of *An. gambiae s.s.*

Most parts of *J. curcas* plant is reported to be toxic (Kumar and Sharma, 2008), probably explaining the larvicidal activity shown in the present study. However, most of the toxic components are contained in the seeds and include saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid and phorbol esters (Kumar and Sharma, 2008). *Jatropha* phorbol esters, particularly, are shown to exhibit insecticidal activities over a wide range of insects, suggesting their potential role in the control of disease vector mosquitoes (Adebowale and Adedire, 2006). Hence, in the present study, the larvicidal activity of the crude methanol leaf extract of *J .curcas* and its column chromatographic may be due to the presence of phorbol esters as toxic constituents. This is possible because phorbol esters of *J.curcas* affect protein kinase (PKC) that is involved in signal transduction and development process of most cells and tissues (Goel et al., 2007). However, the relatively lower larvicidal activities shown in the present study may indicate that the toxic phorbol esters are highly accumulated in *J. curcas* seeds, rather than in other parts of the plant including the leaves. On the other hand, leaves of *J. curcas* are reported to contain bioactive compounds including cyclic triterpenes stigmasterol, campesterol, sistosterol, flavonoids apigenin, vitexin and isovitexin (Kumar and Sharma, 2008) that may jointly or independently contributed to the larvicidal activities of the plant materials against the

late third instar larvae of *An. arabiensis* in the present study. No publicized reports were encountered on the toxicity of *Jatropha* phorbol esters on non-target aquatic organisms, suggesting their potential role in the control of disease vector mosquitoes. Besides, *Jatropha* phorbol esters can be subjected to various chemical and physical treatments that render them harmless to non-target aquatic organisms. Furthermore, some aquatic organisms are known to nullify the toxic effects of *Jatropha* phorbol esters (Goel et al., 2007).

6. CONCLUSIONS AND RECOMMENDATIONS

The present study revealed the larvicidal effects of crude methanol leaf extract of *J. curcas* and its column chromatographic fractions against the late third instar larvae of *An. arabiensis*, the major vector of malaria in Ethiopia. The following conclusions are made based on the findings of the study.

Column chromatographic fractions of methanol leaf extract of *J. curcas* showed better larvicidal activities than the crude extract, suggesting that the larvicidal activity of the crude extract is not due to the synergistic effects of the column chromatographic fractions. Crude methanol leaf extract of *J .curcas* had similar larvicidal activity to 0.5 ppm Temephos at test concentrations ranging from 125 -1000 ppm while Column chromatographic fractions *(F_1* and *F_2*) of crude methanol leaf extract of *J.curcas* showed similar larvicidal activities to 0.5 ppm Temephos at 62.5 and 125 ppm test concentrations. Similarly, *F_3* showed similar larvicidal activity to 0.5 ppm Temephos at 125 ppm test concentration.

Studies must be conducted to identify chemical ingredient/s for the mosquito larvicidal activities in the column chromatographic fractions of crude methanol leaf extract of *J. curcas*. Future wide scale studies need to focus on the larvicidal activities of crude methanol leaf extract of *J. curcas* and its column chromatographic fractions against *An arabiensis* mosquitoes in Ethiopia. Future field studies are also recommended so as to determine the residual activities of the methanol leaf extract of *J.curcas* and its effects on non-target organisms.

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