Evaluation of Larvicidal Activity of Leaf Extract of *Lantana camara* (Family: Verbenaceae) Against the *Aedes aegypti* Mosquito

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Abstract

Aqueous and ethanolic leaf extracts of Lantana camara were prepared and four different concentrations (0.3, 0.6, 0.9 and 1.2 g/ l) of both extracts were tested for larvicidal activity against the fourth instar larvae of Aedes aegypti for 24 hours in the laboratory. The 24-h percentage mortality rates ranged from 91.66 to 96.66 % in larvae treated with 0.3 to 1.2 g/L ethanolic L. camara leaf extract, respectively. The 24-h lethal time (LT_{50}) and lethal concentration (LC_{50}) were 6.8 h and 0.48 g/l, respectively. The aqueous extract did not result in more than 35 % larval mortality. The 24-h percentage mortality between the treatment groups were significantly different (P < 0.001) and also when compared with the control (P < 0.05). The result showed that the ethanolic extract was more lethal than the aqueous extract to A. aegypti larvae. The ethanolic extract also limited the pupation and the adult emergence that was inhibited by the ethanolic extract at 0.9 and 1.2g/L extract.

Keywords: *Aedes aegypti, Lantana camara,* Leaf extract, Larvicide, Pupation, Emergence

Introduction

In Nigeria, *Lantana camara* is commonly used in horticulture as a hedge around homes and lawns and in the Indian sub-continent, it is considered as an invasive weed in plantations (Bor, 1953). In ethno-medicine, Kodwara (1976) reported that the plant is used in the treatment of several diseases like sore throat, conjunctivitis, cough, toothache, cold, high blood pressure and as an antiseptic in cuts and sores.

The age-long war waged on mankind by the insects, particularly the mosquitoes, has continued to challenge the ingenuity of man in devising effective control measures against insect vectors of human diseases such as malaria, filariasis, encephalitis and yellow fever. Thus, the mosquitoes pose serious health problems to man and his life stock. *Aedes aegypti* mosquito is a vector of yellow fever, dengue fever (Gubler, 1997) and the epidemic of these diseases particularly the yellow fever, has been reported in Nigeria (Nasidi *et al.* 1989). So far, the control strategies for mosquitoes have over the years depended on the use of synthetic insecticides with its attendant human public health implications and toxic effect on non-target organisms. It has also caused serious environmental concerns (Hayes and Laws, 1991). Other problems associated with these synthetic insecticides included but not limited to, high cost (Jackal, 1993), development of resistant species (Brown, 1986), human toxicity reactions (Liu *et al.* 2003) and non-compatibility with integrated pest management protocols (Schmutterer, 1990). The use of these insecticides is also limited by non-availability in the rural areas where over 80 % of the people live in Nigeria.

The problems associated with the use synthetic insecticides have necessitated the search for cheap, safe, environmentally friendly phytochemically bioactive insecticides that are readily available. Hence, Mohinder (2001) reported that garlic extracts prevented the hatching of the eggs of *A. aegypti*. The leaf and bark extracts of *Cryptomeria japonica* (Cheng et al.2003) and *Murraya koenigii* (Harre and Kamath, 2004) were reported to have high larvicidal activity against *Aedes aegypti.* Also, extract from neem seed kernel was reported to have high larvicidal toxicity against *A. aegypti* larva (Sukumar *et al*.1991, Umar *et al*.2006) and *Culex* mosquito (Rao *et al*.1992; Mmangi and Mukiama, 1988).

Since mosquitoes represented one ubiquitous group of insects waging a relentless war against humanity, the search for low-cost and readily available phytochemical alternatives to synthetic insecticides will continue to generate robust interest from both researchers and policy makers. Our interest in the plant was elicited when we found that fresh leaves of *Lantana camara* repelled and / or was lethal to mosquitoes and other insects that flock around electric bulbs in homes. We also observed that mosquito bite was prevented when fresh leaves were kept near

the body, thus suggesting that the plant may have insecticidal activity. Therefore, the purpose of this study was to investigate the larvicidal activity of leaf extracts from *Lantana camara* against *Aedes aegypti* mosquitoes.

Materials and Methods

Preparation of *Lantana camara* leaf extracts: The *L. camara* leaves were collected from the University of Nigeria, Nsukka and identified in the Department of Botany herbarium. The ethanolic and aqueous extracts were prepared and used for the study. The ethanolic extracts were obtained after the fresh leaves were air-dried at room temperature and ground into a fine powder using Thomas Willey mill. 200g of the ground dried leaves were soaked in 300ml ethanol for 48 hours in an air tight 500-ml glass container. Thereafter it was filtered with Wathman No 1 filter paper and was concentrated with vacuum evaporator at room temperature and stored in a deep freezer until when used. The aqueous extract was prepared by mashing 200g of freshly collected leaves in 500ml of water. This was filtered using Wathman filter paper No 1 and concentrated in a water bath. The concentrated extract was stored in a deep freezer at 4^{o}C until when used. The percentage yields of the ethanolic and aqueous extracts were 10.33 and 8.33 %, respectively.

Phytochemical screening of the leaf extract: The phytochemical assay was carried out according to the procedures of Harborne (1973), Trease and Evans (1989) as described in Khanna and Kannabiran (2007).

Collection of the *Aedes aegypti* egg: The mosquito eggs were obtained with the assistance of Mr. Godwin Ngwu, the Senior Technologist in the Department of Zoology as egg rafts. A 1L glass container was used as the oviposition site. The eggs were found to hatch within 16h and in about 4-5 days the larvae developed into the fourth instar larvae which were used for the study.

Larvicidal studies: The larvicidal bioassay system consisted of 500- ml beakers containing 250 ml of each test concentration. Four different concentrations (0.3, 0.6, 0.9 and 1.2 g/l) of both the ethanolic and aqueous extracts were prepared and tested on the fourth instar larva of *A. aegypti* for larvicidal activity. Each treatment group consisted of three replicate experiments that contained twenty larvae each. A fifth experimental group containing water only, served as the control. The bioassay systems were covered with a fine mesh screen to prevent the entry of any predator or escape of any adult mosquito. At 6-hourly intervals, the mortality in each replicate experiment was recorded for 24 hours.

Statistical analysis: After 24h, the percentage mortality was calculated by correcting for natural mortality (control) using Abbott's formulae as described by Ndione *et al.* (2007) as follows: % Mortality = [(TM-CM) ÷ (N-CM)] x 100, where TM= mortality in the test concentration, CM= mortality in the control group, N = total no of test organisms in the each treatment.

The LC_{50} (median lethal concentration) values of both the ethanolic and aqueous extracts were calculated using a probit plot of log concentration and percentage mortality (Finney, 1971). The median lethal time (LT_{50}) when 50 % of the larvae died within the 24-h study period was calculated by plotting the percentage mortality against time. The differences between treatments were analyzed using one-way analysis of variance. This was followed with a Student's Newman-Keuls post-hoc test.

Results

Phytochemical properties: The result of the qualitative phytochemical studies of the *L. camara* leaf extract is shown in Table 1. The cyanogenic glucosides were not identified in either the ethanolic or aqueous extracts. The alkaloids were present in high proportion in both extracts. With the exception of the tannins and resins found in the ethanolic extracts, both types of extracts contained similar qualitative phytochemical profile.

Larvicidal activity: The result of the study showed that both the ethanolic and aqueous leaf extracts of *L. camara* have larvicidal activity against *A. aegypti* mosquito. Throughout the period of study, there was no mortality in the control group. The treatment of the 4^{th} instar larvae with ethanolic leaf extract resulted in more than 90 % mortality (Table 2).

A 24-h exposure of the fourth instar larvae of *A. aegypti* to 0.3 and 0.6 g/ l ethanolic extract of *L. camara* resulted in 91 % mortality. When the larvae were exposed to 0.9 and 1.2 g/l ethanolic extracts, the percentage mortalities were 93.3 and 96.6 %, respectively. The mortality rates increased with ethanolic extract concentration and with duration of treatment.

Table 1: Qualitative analysis of phytochemicals of leaf extracts of *Lantana camara*

Phytochemical	Ethanolic extract	Aqueous extract
Cyanogenic glucosides	Nd	nd
Alkaloids	+ + +	+ + +
Glycosides	+ +	+ +
Reducing sugars	+ +	+ +
Flavonoids	+ +	+
Resins	+	-
Steroids	+ +	+ +
Tepenoids	+ + +	+ +
Tannins	+ +	-
Acidic compounds	-	-
Saponins	+ + +	+ + +
Carbohydrates	+ + +	+ + +

Nd =Not detected; - = absent; += present in small amount; ++=present in moderate high concentration; +++ = present in in very high amount; ++++ =abundantly present.

In the groups treated with aqueous leaf extract (Table 3), the mortality rates were 8.3 and 15 % in the groups exposed to 0.3 and 0.6g/L extracts, respectively after 24h exposure. When the larvae were exposed to 0.9 and 1.2 g/L aqueous leaf extracts for 24 h, the mortality rates were 26 and 35 %, respectively. Generally, in the group treated with aqueous extract, there was no lethality in the first 6 hours.

The LC_{50} of the ethanolic extracts were 0.92, 0.65, 0.51 and 0.48 g/ L at 6, 12, 18 and 24 h of exposure times, respectively. This is an indication that the lethality increased with duration of exposure. The LT_{50} (median lethal time) of the ethanolic extract was 6.7 h. The mortality rates in the treatment groups were significantly different ($F= 5.18$, df 4, 10; $P < 0.05$). The LC_{50} and LT_{50} of the aqueous extracts could not be calculated because the mortality rate did not exceed 35 %.

Percentage pupation and adult emergence: The effect of both the ethanolic and aqueous leaf extracts on the pupation and adult emergence is shown in Table 4. The pupation and adult emergence were 100 percent in the control experiment. The pupation rate decreased from8.3 to 3.3 % in the 0.3 and 1.2 g/L ethanolic extracts, respectively. The adult emergence was 2.1 and 1.0% in the 0.3 and 0.6g/L ethanolic extract, respectively. At higher concentrations of the ethanolic extract, the adult emergence was nil. In the aqueous extract the pupation decreased from 91.6% to 65.0% in the groups treated with 0.3 and 1.2g/L extract, respectively. Similarly, adult emergence was highest in the group treated with 0.3g/L extract and least (56%) in the group exposed to 1.2g/L extract. The aqueous extract had no significant effect on pupation and adult emergence. The rates of pupation ranged from 3.3-8.3 in the groups treated with 1.2 and 0.3g/L ethanolic extracts of *L. camara.*

Discussion

Research into the possible use of phytochemically bioactive compounds in the control of mosquito vectors of human diseases has become imperative due essentially to the resistance of mosquitoes to synthetic insecticides besides other problems like human toxicity and environmentally related ones.

The results of this study are consistent with earlier reports that showed that some plant extracts have insecticidal activity. Dwivedi and Garg (2003) reported that flower extracts of *L. camara* caused 87.32 % population reduction in the rice moth, *Coryra cephalonica* by suppressing the adult emergence. Similarly, Bouda *et al.* (2001) reported that oil from *L. camara* caused 100 % mortality in *Sitophilus zeamais* at a concentration of 0.5 % (v/w). In this study, the ethanolic extract caused more than 90 % mortality in the test concentration. The rate of adult emergence in *A. aegypti* exposed to 0.3 and 0.6g/L ethanolic extracts were 2.1 and 1.0, respectively. At higher concentrations, no adult emergence was recorded. Mohammed and Chadee (2007) also reported that aqueous extracts of *Mangifera indica* (C1), *M. indica* (C2) and *Cordio curassavica* as well as *Azadirachta indica* were toxic to *Ae. aegypti* larvae. Rodriques *et al.* (2006) reported that over 65 % mortality in *A. aegypti* larvae exposed to plant extracts of *Annona cassiflora, Serjonia lethalis* and *Xylopia aromatica* for 24 h

Studies with neem seed extracts (Jacobson, 1981; Shmutterer, 1990; Sivagnaname and Kahyanasundraram, 2004; Umar *et al*., 2006) showed that it has strong larvicidal effect on *A. aegypti* and culex mosquitoes (Rao *et al.* 1992). Our results are also consistent with the report of Cheng et al. (2003) that demonstrated that leaf and bark extracts of *Cryptomeria japonica* had high larvicidal activity against *A. aegypti.* It is also in agreement with the findings of Harre and Kamath (2004) that extracts of *Murraya koeigii* and *Coriandrum satirum* were effective against *A. aegypti* larvae and culex mosquito. Rodriques *et al.* (2006) reported that extracts of cerrodo plants exhibited larvicidal activity against *A. aegypti*.

Table 2: Percentage mortality of *Ae. aegytpi* mosquito larvae treated with different concentrations of ethanolic leaf extracts of *L. camara* for 24 h

Test Concentration (g/L)	Mortality (%)			
	6 h	12h	18h	24h
0.0	0±0	0±0	0±0	0±0
0.3	41.7±2.8	71.6±1.4	86.6±1.8	91.6±1.0
0,6	35.0±1.0	56.7±1.4	81.6±1.5	91.6±1.7
0.9	51.6±2.1	65.0±1.3	83.3±3.2	93.3±1.8
1.2	53.3±1.6	73.3±2.4	90.0±3.1	96.6±2.0

Values are mean (n=3±sd).

Table 3: Percentage mortality of *Ae. aegytpi* mosquito larvae treated with different concentrations of aqueous leaf extracts of *L. camara* for 24 h

Concentrations (g/L)	Mortality (%)			
	6h	12h	18h	24h
0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
0.3	0.0±0.0	1.7±0.0	6.6±1.0	0.03±0.00
0,6	0.0±0.0	3.3±0.0	11.7±1.6	1.3±0.0
0.9	0.0±0.0	6.6±0.0	13.3±1.4	1.3±0.0
1.2	0.0±0.0	11.6±1.1	16.7±2.2	20±1.5

Values are mean (n=3±sd).

Table 4: Percentage successful pupation and adult emergence of the 4th instar larvae of *A. aegypti* exposed to ethanolic and aqueous extracts of *L. camara*

Test Concentration(g/L)	Ethanolic Extract		Aqueous Extract	
	Pupae (%)	Adult (%)	Pupae (%)	Adult (%)
Control	100	100	100	100
0.3	8.3	2.1	91.6	83.1
0.6	8.3	1.0	85	66.7
0.9	6.6	0	73.3	60.0
1.2	3.3	0	65	56.7

Table 5: LC 50 value of some plant extracts for mosquito control based on larvicidal activity after 24h exposure

Species	Plant	Extract	24h LC_{50}	Reference
A. aegypti	*Quillaja sapanin*	Plant	500mg/L	Wiemana & Bishnu (2003)
A. aegypti	*Atlanda monophylla*	Plant	5.7mg/L	Sivagnanama & Kalyanasundanm (2004)
A. aegypti	*Ageratina adenophora*	Leaf	345.34ppm	Mohan & Ramaswamy(2007)
A. aegypti	*Momrdica charantia*	Crude fruit	1.45%	Singh et al(2006)
A. aegypti	*Formulated neem oil*	Neem oil	8mg/L	Ndione et al(2007)
Culex pipens	*Balanites aegyptica*	Root	2%	Bishnu & Wiesman (2005)
Anopheles stephensi	*Momrdica charantia*	Crude fruit	.50%	Singh et al (2006)
A. aegypti	*Lantana camara*	Ethanolic leaf extract	0.48g/L	This study

Similarly, Jang et al. (2005) reported that extracts of *Chaemecyparis obtusa* leaves had larvicidal activity against *A. aegypti* and other mosquitoes.

The lethality effectiveness of plant extracts against mosquito larvae and adult vary from species to species and depends on the insect. Minijas and Sarda (1986) had earlier reported that extracts of *Swartzia madagasriensis* fruit was more effective against *Anopheles gambiae* and *A. aegypti* and had no effect on *Culex quinquefasciatus* larvae. Similarly, Sujatha *et al.* (1988) observed that extracts of *Citrus medica* was

lethal to *Anopheles stephens* larvae while the extract of *Madhuca longifola* was ineffective.

Essential oils of plant origin have also proved to be effective control agents against both the larvae and adult mosquitoes. Tawatsin *et al.* (2006) reported that oils extracted from guava and fingeroot rhizomes provided 100 % protection against mosquitoes for 9 h thereafter the repellant efficacy decreased to 80 % after 11 h. Also, Lee (2006) reported that essential oils extracted eleven medicinal plants caused 100% mortality to *A. aegypti, Culex pipens pallens* after 24 h post exposure period to 200 and 100 ppm extracts. They further reported that at lower concentration of 25 ppm extracts from *Citrus bergama, Commophora myrrha* and *Pimenta racemosa* resulted in 100 % mortality of the mosquitoes while at 50 ppm oils from *Citrus bargamia, C. cyminum* and *Dacus carota* caused 100 % mortality against *Ae aegypti* and *Culex pipens pallens*. Also, oils of spearmint *Mentha spicata* var. *viridis* rich in piperithenone oxide was found to be effective against *Anopheles stephensi* as it inhibited the hatching of the egg (Khanuja *et al.* 2001, Tripathi *et al*. 2004) and showed broad spectrum activity on insects. Similarly, Zhu *et al*. (2006) demonstrated that oils from eucalyptus, cinnamon and amyris caused more than 80 % mortality in the larvae of *A. aegypti* at concentration of 80- 320µg/ml.

The result of this study further demonstrated that the ethanolic extract had far greater larvicidal potency than the aqueous extract. This may indicate that the active larvicidal components are better extracted with ethanol since the qualitative phytochemical profiles of both the ethanolic and aqueous extracts are similar in most regards. Thus, our observation that the ethanolic extract of *Lantana camara* was more potent than the aqueous extract is consistent with the report of Shaalan *et al*. (2006) that the kind of extraction solvent has significant effect on the potency of plant extracts. According to Mulla and Su (1999), the polarity of the solvent also affects the plant extract potency. Hence,Moawed (1998) ethanolic extract of *Haplophyllum tuberculatum* and *Kuta graveolens* were more efficacious against *culex pipiens* than the petroleum ether extract. Similarly, Rodriques et al. (2006) reported that ethanolic extracts of *Annona crassiflora, Serjania lethalis* and *Xylopia aromatica* were more lethal than the hexane extract. Also, Jang et al. (2005) reported that methanolic extract of *Chamecyparis sp* was effective whereas the aqueous extract had no larvicidal activity. Besides larvicidal potency, plant extracts are known to adversely reduce the fecundity of *A. aegypti* (Muthukrishnan and Pushpalatha, 2001). The LC 50 value of 0.48g/L of the ethanolic leaf extract *L. camara* shows that it has higher larvicidal against *A. aegypti* mosquito than some other tested plant-based products (Wiesman and Bishnu, 2003; Sivagnanma and Kalyanasundaram, 2004) (Table 5).This promises to open windows of opportunity for effective and affordable approach to the control of the yellow fever vector in Nigeria since the plant is readily available in both the rural and urban centres.

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