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Toxicity and Repellency Action of *Lantana camara* on *Annopheles gambiae*

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Abstract
Larvicidal, Adulticidal and repellency of Lantana camara was investigated against Anopheles gambiae. 2nd instar larvae and adult of Anopheles gambiae were exposed to serial dilution of the stock solution in 10, 20, 30, 40 and 50%v/v of hexane and ethanol extracts of L camara. The control experiment contained distilled water only. Mortality rate of the larvae and adults were calculated after every 24hrs for three days. Repellency and knockdown action was determined using improvised mosquito coil. Hexane and Ethanol extracts were effective against An gambiae. Hexane extract had a higher larvicidal effect while ethanol had a higher adulticidal and repellency effect than hexane. An gambiae was more susceptible to hexane than ethanol extract of L. camara. It was 2.41(24hours), 3.20 (48 hours) and 1.96 (at 72hours) times more susceptible than ethanol. Protection (repellency) increased with increase in concentration of both hexane and ethanol extracts of L. camara. 100% protection was achieved with 100% v/v concentration of both hexane and ethanol respectively. For the coil, percentage knockdown increased with increase in concentration of L. camara powder. Highest knockdown 90% was also achieved with 100% concentration of L. camara coil
Keywords*: Adulticidal, larvicil, repellency, mosquito coil, hexane extract, ethanol extract, Lantana camara, Anopheles gambiae*

Introduction

Malaria is a mosquito borne infectious disease of humans and other animals caused by a single celled protozoan parasite*Plasmodium. Anopheles gambiae* transmits *Plasmodium falciparum,* which is the most severe of the four malarial agents. There are an estimated 300 to 500 million cases of malaria each year and as a result, 1.5 to 2.7 million deaths occurs worldwide. Malaria is a major cause of infant mortalityin tropics and sub-tropics regions of the world. It is the only insect borne parasitic disease comparable in impact to the world's major killer transmissible diseases: diarrhea, acute respiratory infections, tuberculosis and AIDS (Denloye *et al*., 2003).

Synthetic insecticides have been used to control mosquitoes over time, but this leads to adverse repercussions such as poisoning, environmental destruction and development of resistance in target species (Don-Pedro, 1980; Boroffice and Borofice 1993). These problems have necessitated the need for search and development of environmentally

safe, biodegradable, low cost, indigenous methods for malaria vector control which can be used as an alternative to synthetic insecticides (Balandrin *et al*., 1985).

The most important of alternatives to synthetic insecticides is Phytochemicals (plant extracts) with a broad range of activities against mosquitoes such as ovicidal, larvicidal, adulticidal, oviposition deterrence, developmental toxicity, antifeedants, repellents, hatching and emergence blockers (Bakkali *et al*., 2008, Sukumar, 1991).Though lots of research works have been carried out against natural products on mosquitoes (Bowers *et al*., 1995, Denloye *et al.*, 1995, 2003, Dua, 2010). There are still lots of medicinal plant products that are yet to be tested fortheir potency against the control of mosquitoes.

Lantana camara, also known as big sage (Malaysia), wild sage, red sage, white sage (Caribbean) and tick berry (South Africa) and Elepo (in Yoruba:Nigerian language) is a species of flowering plant within the family Verbenaceae. The stalks of *L. camara* have been used in the construction of furniture such as chairs and tables, however, the main uses have historically been medicinal and ornamental. Studies show that *L. camara* has antimicrobial, fungicidal and insecticidal activity against stored grain insect pests, vegetable crops pest, mosquito larvae, repellent, and other biological activities .*L. camara* has also been used in traditional herbal medicines for treatment of ailments, including cancer, skin itches, leprosy, rabies, chickenpox, measles, asthma and ulcers (Balandrin *et al*., 1995).

The aim of the study is to evaluate the repellency and larvicidal effect of hexane and ethanol extracts of *Lantana camara* against adult and larvae *Anopheles gambiae* mosquito respectively and the repellency effect and knockdown action of dried powdered extract of the leaves as impoverished coil on adults of *Anopheles gambiae*. Finally, to determine which extract (hexane and ethanol) has a higher toxicity effect on *Anopheles gambiae*.

Materials and Methods

Source and Preparation of Test Plant Species

Fresh leaves of *Lantana camara* were obtained from ornamental gardens in University of Lagos (UNILAG) (Plates1and 2). The specimen of the plant was identified as *L. camara* UNILAG herbarium.

The leaves were washed under running water and air dried at room temperature on laboratory tables for 7days. The dried leaves were pulverized using an electronic blenderand thereafter, stored in air tight polythene (Ziploc bags®) bags until needed.

Plate 1*Lantana camara* plant with its yellow flower from University of Lagos

Plate2 Cluster of black berry fruits of *Lantana camara* from University of Lagos

Extraction of Hexane and Ethanol Extracts of *L. camara* Leaf Powder

Ethanolic and hexane extracts of *L. camara* were carried out on the leaf powder. 10 g of dried *Lantana camara* leaf powder was steeped in 150 ml of ethanol in a 250 ml conical flask properly cocked to avoid evaporation for 24 hours at room temperature. The mixture was stirred thoroughly and filtered through Whatman filter paper size 10. The filtrate was kept in Amber bottles until needed. The procedure was repeated several times to obtain 250 ml for each crude extract. It was labeled and stored for use in bioassay studies. Same procedure was used for hexane extraction of *Lantana camara.*

Preparation of Mosquito Repellent Coil from *L. camara* Leaf Powder

Insecticidal repellent coil were prepared from the leaf powder of *L. camara* that has been dried at room temperature. The leaves were pulverized with pestle and mortar and further pulverization into a powder form was achieved with a Kenwood electric kitchen blender. Different concentrations of the coil were achieved by moulding with saw dust as inert material blended with starch made from processed cassava as binder. Concentrations of 20, 40, 60, 80 and 100% were made in ratio formula by adding 2 g of the test plant with 18 g of saw dust to obtain 20%; and graduated ratio for 40, 60, 80 and 100%. Moulded coils were dried in the laboratory table laid with foil paper (Plate 3) at room temperature. Moulded saw dust shavings bounded with processed starch from cassava was used for the control treatment.

Plate3: A picture showing molded coil of *Lantana camara leaf powder*

Larva and Adult Rearing

Anopheles gambiae larvae were collected from the National Institute of Medical Research (NIMR) Yaba, Lagos. The larvae (over 200) were placed in plastic containers (5 plastic containers of 5 litres each) that contained yeast powder in water until they emerged into adults. The adults were used for the adulticidal experiments. The emerged adults were periodically blood fed on volunteer's arms dipped into the cage through the netting and were also were provided with 10% glucose solution on cotton wicks to feed on. The cotton was kept moist and changed every day.

Toxicity of the Ethanol and Hexane Extract of *L. camara* on *An. gambiae* Larvae

Ten (10) 2nd instar *Anopheles gambiae* larvae were transferred into a transparent disposable cup. The larvae were then exposed to serial dilution of the stock solution in 10, 20, 30, 40, 50% v/v. The control experiment contained distilled water only. The mortality rate of larvae was recorded after every 24hrs for three days at ambient temperature and relative humidity. This procedure was replicated three times. Percentage mortality as calculated thus;

$$\frac{\text{Total number of dead larvae}}{\text{Total number of larvae}} \times 100$$

Repellency and Knockdown Effect of Ethanol and Hexane Extract of *Lantana camara* Leaf Powder against Adult *Anopheles gambiae*

The adult *Anopheles gambiae* used in the test were 2 days old. Two cages were used and ten mosquitoes were put in each cage. The crude extract was serially diluted with distilled water at 20, 40, 60, 80 and 100% concentrations. Control was also set using only distilled water. The arms of the volunteer were initially washed with toilet soap

and distilled water. Ethanol or hexane in cotton wool was used to clean the volunteer's arms to prevent the mosquito's attractiveness to the sweat of the arm. One of the cleaned arms of the volunteer was rubbed with each level of concentrations of *Lantana camara* leaf powder at each time of treatment application while the second hand was rubbed with distilled water (control). The two arms were each exposed to ten 0 - 2 day old unfed adult mosquitoes at thirty minutes interval.

The repellency of the different concentrations of the extract was measured on the basis of the number of bites received within thirty minutes on both arms. After each exposure for the different concentration levels, the arm was thoroughly washed and cleaned with ethanol (for ethanol extract) and hexane (for hexane extract) before another dosage was introduced on the arm into another cage. New unfed adult mosquitoes were used for each concentration levels.

The *Anopheles gambiae* adult used in the test was 0 - 2 day old. Two cages were used and ten mosquitoes were put in the cage each. For the coil, varying concentrations of 20, 40, 60, 80 and 100% and control were molded; mosquitoes were exposed to smoldering coil and repellency at different concentration was recorded every 30 minutes. The repellency of the different concentration of the coil was measured on the basis of the number of bites on the arms within thirty minutes while the knockdown effect was calculated on the number of insects that lay (fallen down) on the floor of the cage.

The percentage repellency and knockdown effect was calculated as thus:

$$\frac{\text{Mean no of bites received in control area (arm)} - \text{mean number of bites received in treatment area (arm)}}{\text{Mean number of bites received}} \times 100$$

or and for the knockdown effect:

$$\frac{\text{Mean no of fallen insects in the control area} - \text{mean number of fallen insects in the treatment area}}{\text{Mean number of fallen insects}} \times 100$$

Statistical Analysis

LC_{50} for 24, 48 and 72 hours (for larvae) was calculated using the Probit analysis after Finney (1971). The control mortalities were corrected by using Abbott's formula (Abbott's 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

The LC_5, LC_{50}, LC_{99} and 95% upper confidence limit (UCL) and lower confidence limit, regression and chi-square values were calculated by using Probit analysis (Finney 1971).

Data were subjected to statistical analysis using SPSS 20. The different levels of concentration of hexane, ethanol, repellency and knock down at various times were analyzed separately using analysis of variance (ANOVA). Means were compared using Duncan's Multiple Range Test at 0.05% level of significance.

Results

Effect of hexane and Ethanol Extract of *L. camara* Leaf Powder on *An. gambiae*

Larval mortality of *An. gambiae* increased with increase in concentration in both extracts as well as the exposure time (Tables 1 and 2).Larval mortality with hexane extract ranged from 73.3 at 24hours to 100.0% at 72hours (Table 1) and from 53.3 to 80.0% respectively with ethanol extract (Table 2). All the larvae (100%) at 30 to 50% v/v hexane extract died after 72hours and, were significantly higher than mortality in control and 10%v/v hexane extract (Table 1). Mortality in ethanol extract was 80 to 93% for adult but larvae died at same concentration and were also significantly higher than control and 10% v/v extract.

Table 1: The toxicity effect of hexane extract of *Lantana camara* leaf powder on larvae of *Anopheles gambiae*

Concentration (%v/v)	Time of Exposure		
	24 hours	48 hours	72 hours
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
10	33.33 ± 3.06^{ab}	53.33 ± 1.15^{b}	80.00 ± 2.00^{b}
20	40.00 ± 3.46^{ab}	60.00 ± 2.00^{bc}	86.67 ± 1.15^{bc}
30	66.67 ± 2.31^{b}	86.67 ± 1.15^{cd}	100.00 ± 0.00^{c}
40	80.00 ± 2.00^{b}	93.33 ± 1.15^{d}	100.00 ± 0.00^{c}
50	73.33 ± 2.31^{b}	86.67 ± 2.31^{cd}	100.00 ± 0.00^{c}

Mean values with different subscript on the same column show significant differences at $p<0.05$. (Df = 5)

Table 2 The toxicity effect of ethanol extract of *Lantana camara* leaf powder on larvae of *Anopheles gambiae*

Concentration (%v/v)	Time		
	24 hours	48 hours	72 hours
Control	0.00±0.00[a]	0.00±0.00 [a]	0.00±0.00[a]
10	0.00±0.00[a]	6.67±1.15[a]	46.67±2.31[b]
20	6.70±1.15[a]	26.67±2.31[a]	60.00±2.00[bc]
30	20.00±3.46[ab]	60.00±3.46[b]	86.67±1.15[cd]
40	40.00±1.00[bc]	60.00±1.15[b]	93.33±1.15[d]
50	53.33±1.15[c]	60.00±1.51[b]	80.00±2.00[cd]

Mean values with different subscript on the same column show significant differences at $p<0.05$. (Df = 5)

Mortality Rate of Hexane and Ethanol Extract of *Lantana camara* Leaf Powder on *Anopheles gambiae* Larvae

Mortality rate of hexane and ethanol extracts of *L. camara* is shown in figures 1 to 6. In both extracts, mortality rises, gets to the peak then drops (Figures 1,3,4,6) or remains constant (Figure 5).

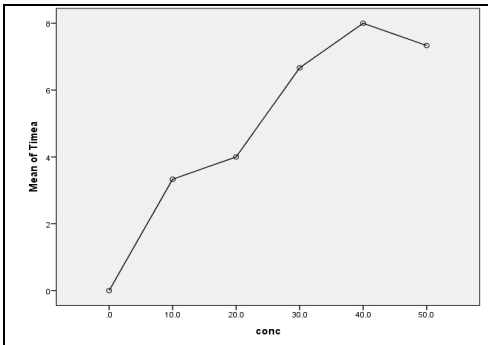

Figure 1: Mortality rate of hexane extract of *Lantana camara* within 24hrs.

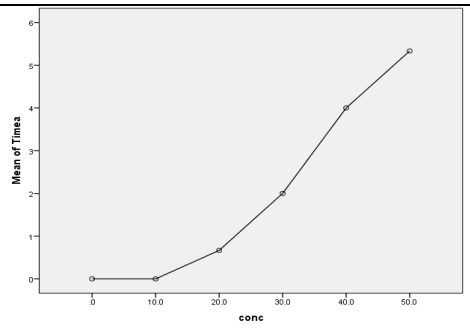

Figure 2:Mortality rate of ethanol extract of *Lantana camara* within 24hrs

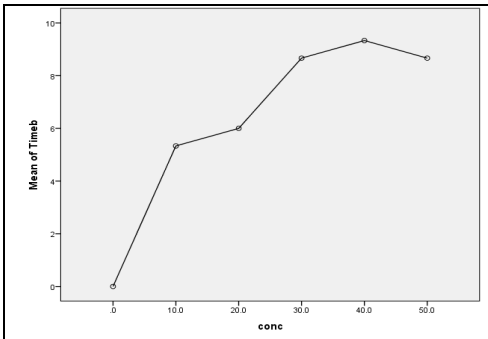

Figure 3: Mortality rate of hexane extract of *Lantana camara* within 48hrs

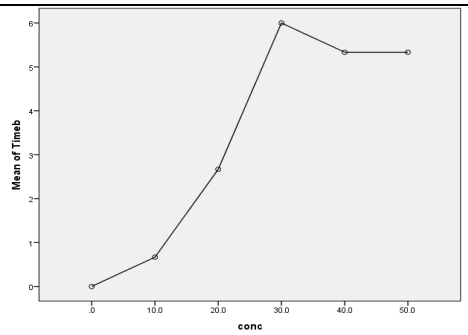

Figure 4:Mortality rate of ethanol extract of *Lantana camara* within 48hrs

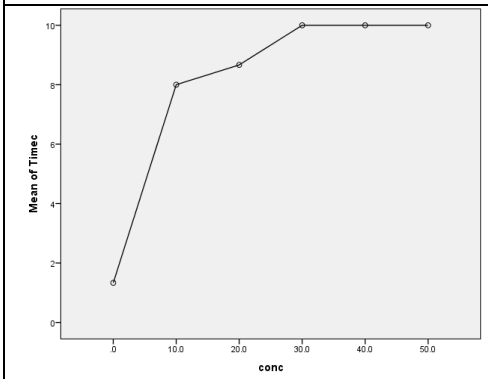

Figure 5:Mortality rate of hexane extract of *Lantana camara* within 72hrs

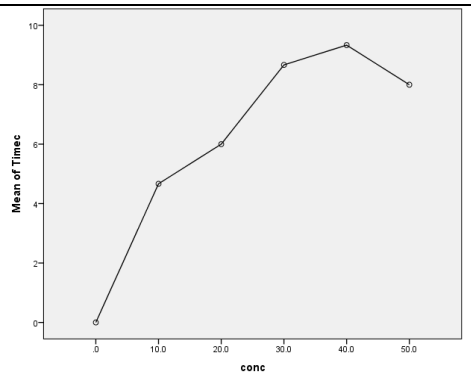

Figure 6: Mortality rate of ethanol extract of *Lantana camara* within 72hrs

LC_{50} Values of Hexane and Ethanol Extracts of *Lantana camara* Leaf Powder on Larvae of *Anopheles gambiae*

The LC_{50} value of hexane on *Anopheles gambiae* larvae was significantly lower than that of ethanol extract. It ranged from 5.773 (72hours) to 19.493 (24hours) for hexane extract and from 11.294 (72hours) to 46.981 (24hours) for ethanol extract (Table 3-5). The toxicity factor also shows that *Anopheles gambiae* is more susceptible to hexane extracts than ethanol extract of *L. camara* leaf powder at the following differing ratios: 2.4 (24 hours) > 3.20 (48 hours) > 1.96 (72 hours).

Table 3: LC_{50} values of hexane and ethanol extract of *Lantana camara* Leaf powder on *Anopheles gambiae at* 24 hrs.

Test botanical	Duration	LC_{50}	Confidence interval	Regression equation	Slope ± S.E	DF	TF
Hexane	24hrs	19.493	12.978-25.286	Y= -2.402+(-3.823)x	-3.823±0.628	3	1.00
Ethanol	24hrs	46.981	40.571-61.051	Y= -7.010+(-4.946)x	-4.946±1.417	3	2.41

Table 4: LC_{50} values of hexane and ethanol extract of *Lantana camara* Leaf powder on the larvae *Anopheles gambiae at* 48 hrs.

Test botanical	Duration	LC_{50}	Confidence interval	Regression equation	Slope ± S.E	DF	TF
Hexane	48hrs	10.173	4.414-14.483	Y= -1.927+(-2.982)x	-2.982±0.646	3	1.00
Ethanol	48hrs	32.543	26.812-41.067	Y= -3.940+(-5.197)x	-5.197±0.758	3	3.20

Table 5: LC_{50} values of hexane and ethanol extract of *Lantana camara* Leaf powder on *Anopheles gambiae* larvae at 72 hrs.

Test botanical	Duration	LC_{50}	Confidence interval	Regression equation	Slope ± S.E	DF	TF
Hexane	72hrs	5.773	1.273-8.648	Y= -2.506+(-2.070)x	-2.070±1.210	3	1.00
Ethanol	72hrs	11.294	5.295-15.771	Y= -1.983+(-3.124)x	-3.124±0.635	3	1.96

Repellency Test of Hexane and Ethanolic Extracts of *Lantana camara* Leaf Powder and Knock Down Effect of Molded Coil on *Anopheles gambiae* Adults After 30 Minutes

Repellency of *L. camara* on adult *An. gambiae* mosquitoes is shown in Table 6. The knockdown effect increased with concentration in both hexane and ethanol extracts of *L. camara* leaf powder. 100% protection was achieved with 100% v/v concentration of both hexane and ethanol extracts, respectively. Highest knockdown effect of 90% of test insect was recorded at 100% v/v concentration (Table 6).

Table 6: The repellency and percentage knockdown effect of of *L camara* on Adults of *An. gambiae* adult

Conc(%v/v)	Repellency (%) Percentage Knock Down		
	Hexane	Ethanol	Coil
Control	0.00	0.00	0.00
20	10.00	30.00	30.00
40	30.00	50.00	50.00
60	50.00	70.00	60.00
80	70.00	80.00	70.00
100	80.00	100.00	90.00

Discussion and Conclusion

The study showed that the crude extracts of *L. camara* leaf powder were effective in controlling the larvae o*f An. gambiae*. This is in agreement with the findings of Nath *et al*., (2006) who found that the leaf extract of *L. camara* showed larvicidal effectiveness against *C. quinquefasciatus* and *Aedes albopictus.* Moreover, Innocent *et al*., (2008) in their study found the root barks extracts of *Lantana viburnoides* to be toxic against 3rdand early 4thinstar larvae of *Anopheles gambiae*. They reported that extracts could serve as a source of larvicides for managing various mosquito habitats in the field even in their semi purified form. The presence of compounds such as lantadene, triterpenoids and furanonaphthaquinones in *Lantana species* maybe the cause of the plant's larvicidal properties on mosquitoes (Nath *et al*., 2006 and Innocent *et al*., 2008).

The extracts from *L. camara* leaves also showed adulticidal activity against *An.* gambiae using hexane (80%) and ethanol (100%) extracts respectively. This is in agreement with Dua *et al* (2010) who found the adulticidal activity of the oil of *L camara* to be most toxic to *An. fluviatilis* followed by *An. culicifacies, C. quinquefasciatus, An. stephensi* and *Ae. Aegypti*. *Moreover,* hexane extract showed a higher adult mosquito repellency and larvicidal effect (100 and 90% respectively) than ethanol extract on *Anopheles gambiae.*

This study also shows that the crude extracts of hexane and ethanol of *Lantana camara* leaf powder are slow-acting botanicals. This is because it was at 72 hours post treatment that the effect of mortality rose up to 100% in hexane and 93.3% in ethanol. While at 24 hours the efficacy of the extracts showed significantly lower mortality of 73.3 and 53.3%, respectively for hexane and ethanol extracts. LC_{50}s of the leaf extracts showed *Lantana camara* to be promising as larvicides, adulticides and repellents against *Anopheles gambiae.*

The use of the plants in insect control offers a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low or no cost at all. L*antana camara* leaves are weed plants that grow around in many places in Nigeria. The leaves can be used as a coil to repel mosquitoes in such areas. Since *lantana camara* is active against insects, less expensive, easily biodegradable to non-toxic products it can be incorporated into integrated vector control.

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