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Research Article

Effects of *Combretum micranthum, Xienmia americana* and *Aloysia citrodora* Leaf Extracts against Oviposition and Egg Viability of *Anopheles gambiae S.L.* (Diptera: Culicidae)

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ABSTRACT

The study investigated the oviposition and egg viability effects of leaf extracts from *Combretum micranthum*, *Xienmia americana*, and *Aloysia citrodora* on female *Anopheles gambiae* mosquitoes. Leaves were sourced from Goron-maje Town, Dambatta LGA, Kano State, and extracts were prepared using ethanol, methanol, and ethyl-acetate. Bioassays were conducted at ambient temperature and relative humidity, testing oviposition activity and egg viability. Ethyl-acetate extracts, particularly from *A. citrodora*, significantly reduced oviposition compared to controls, with a 42.85% decrease. Ethanol extract of *C. micranthum* also showed reduced oviposition (8.84%). Significant differences were observed in the effects of different concentrations (ppm/mL) of plant extracts compared to controls. *X. americana* ethanol leaf extract at 20.0 ml concentration showed a 10% decrease in egg viability, while *Combretum micranthum* extracts at 20.0 ml and 30.0 ml concentrations showed 40.0% and 26.0% reductions in viability respectively. *Aloysia citrodora* methanol extracts exhibited a lower effect (48.0%) on viability compared to controls. *X. americana* ethanol leaf extract at 10% concentration significantly reduced hatching ability (26±06.21). Similarly, ethanol and ethyl-acetate extracts of *C. micranthum* and *A. citrodora* at 20.0 ml and 30.0 ml concentrations showed decreased hatching abilities compared to controls. The study suggests that ethanol and ethyl-acetate extracts of *X. americana*, *C. micranthum*, and *A. citrodora* could be effective and safer methods for mosquito control.

Keywords: *Leaf extracts;* soxhlet*; Oviposition; Egg Viability; Anopheles gambiae s.l.*

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INTRODUCTION

Mosquitoes are nearly ubiquitous and inhabit most regions except Antarctica. They exist in regions more than five thousand metres above sea level and almost one thousand three hundred metres below sea level. Mosquitoes belong to family Culicidae that has about 3500 species belonging to 41 genera (Service, 1986). Only about 100 mosquito species have been implicated as intermediate hosts of vertebrate parasites since 1878 (Foster and Walker, 2002). Malaria is transmitted by female *Anopheles* mosquitoes when they come to bite. About 528 species of *Anopheles* mosquitoes have been described in the world, and approximately 80 of them are important vectors of malaria, filarial nematode and encephalitis virus

Mosquitoes mate only once after emergence from pupae. Females bite hosts to acquire nutrients for egg development followed. Thereafter, oviposition follows and on subsequent blood meal, another set of eggs matures completing a gonotrophic cycle. Females lay 30-300 eggs per oviposition. *Anopheles* mosquitoes lay eggs singly that float on water. Their eggs die when they are out of water. The eggs withstand desiccation and hatch when submerged in water In subfamily *Culicinae,* the *Aedes, Culex, Mansonia, Sabethes* and *Haemagogus* are of most significance. Their eggs form rafts that enable them to float on water surface (Service, 1986).

In Nigeria potential indigenous plants are highly recognized by herbalist as they employ widely for their mosquito repellency properties and vector control. These locally used plant as alternative control of mosquitoes has not been prove on scientific fact for their effects against mosquitoes. For this reasons the current study aim at the use different plants extracts against oviposition and egg viability of female *Anopheles gambiae L.*

MATERIAL AND METHODS

Study Area

The study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Health Science, Al-istiqama University Sumaila Kano.

Collection of *Anopheles gambiae L.*

Anopheles gambiae larvae was obtained from the insectary Unit, Department of Biochemistry, Bayero University Kano.

Processing and Identification of Plant Leaves

Plants leaves of *A. citrodora, C. micranthum* and *X. americaa* were obtained from Goron-maje town, Dambatta LGA, Kano State. The plants were also authenticated by plant taxonomy at the Department of Plant Biology, Bayero University Kano. Healthy leaves of three different plants were washed with tap water, cut into small pieces and air dried. After the plants were completely dry, they were ground into powder by blending. The powdered leaf were as extracted using soxhlet extraction techniques method.

Soxhlet Extraction Process

Soxhlet extraction was conducted based on the protocol of Frank *et al.* (2020). The powdered leaf was place in inside a porous bag (thimble) made up of a clean cloth or strong filter paper and tightly closed. The extraction solvent will be poured into the bottom flask and the thimble into the extractor chamber. The solvent will be place on heating mantle. Each solvent use has it boiling point measured when heating (Frank *et al.,* 2020). The solvent will then be heated from the bottom flask, evaporate and passes through the condenser where it condenses and flow down to the extraction chamber and extract leaf materials by coming in contact. Consequently, when the solvent in the extraction chamber reaches the top of the siphon, the solvent and extraction materials will flow down back into the solvent (Jidong *et al.,* 2009) the entire process continues until the extraction material is completely extracted at a point when solvent flow from extraction chamber does not leaves any residue or becomes colorless. Finally, the extracted leaf material and solvent was transfer into evaporation dish and place on water path at 4 – 45^0c (Frank *et al.,* 2020) for complete extraction of dried leaf materials known as Crude extract.

Rearing Procedure of *An. gambiae s.L*

Healthy emerged adult females and males *An. gambiae* were reared and remain inside the insectary for at least 5days for mating to take place.
Adults was fed with 10% sucrose before then fed with blood meal after 3-5 days (Des *et al.,* 2007). After each

blood meal feeding exercises, successfully fed mosquitoes will become engorge with red colorations abdomen (Clements, 2000) and lay eggs immediately overnight. Beaker was placed inside the cage containing water and a piece of filter paper for oviposition.

Eggs were laid on filter paper over night. Filter paper containing eggs will be placed in a plastic tray with 300ml of distilled water and allowed to hatching into larva (Des *et al.,* 2007). Developing larvae would be fed with pinch yeast every day and the use of clean water is also important for refreshing the environment after every feed. Separate pupa from larvae would be done daily and placed into a plastic bowl for adult to emerge after 2-3days, inside the insectary (Edillot *et al.,* 2007). Colonies would be maintained and all experiments would be carried out at a constant temperature of 25 ± 2ºC and 80 ± 10% relative humidity (Clements, 2000).

Oviposition Activity

The oviposition activity was tested using Rui-de

et al. (2006) method. Gravid female (5-10) day old were put a covered cage and provide with a 10% glucose solution. A serial dilution of plant extract solution at concentrations of 100, 200, ad 300 ppm. Four plastic cups were used, three treated with extract and one without extract (control), were placed in a cage for oviposit. Four repetition were done for each concentration and for each test. Mosquitoes were maintained in condition of 27ºC. The laid eggs were count after 24h by removing the filter paper. Oviposition percentage index was calculated using formulae:

Oviposition rate (%) = Number of eggs in control – Number of eggs in treatment X 100 /

Number of egg in control

Egg Viability:

The methods of Rui-de *et al*. (2000) were followed. Bioassays were conducted at ambient temperature of 29 ± 2 ºC, 80 ± 5 % relative humidity under a photoperiod of 13:11 hour light: dark cycles. Controls were prepared with 100ml of distilled water only. The eggs belonging to the populations of *An. gambiae* were carefully recovered from the rearing containers with fine soft brush each morning, identified and counted under the low power binocular stereo or hand lens. One hundred (100) freshly laid *An. gambiae* eggs were placed into separate 200 ml disposable plastic cups each containing 100 ml of distilled water (WHO, 1996).

Three (3) serial dilutions 10, 20, 30, and 40 mg/ml of ethanol extracts were made from the stock solutions. For treatment, 1ml of each concentration of each extract was added to a series of three cups, with one cup maintained as control received 1ml of distilled water. Treatments were replicated 3 times for each plant extracts. The content of each test cup was stirred gently with a glass rod to ensure homogeneity. Percentage of egg viability was calculated by dividing the number of larvae that emerged from the eggs at every 24 and 48-h after treatment by the total number of eggs laid. WHO (1996) formula was employed to correct percentage viability of eggs if control inhibition of egg hatching.

Percentage of egg viability (%) = Number of larvae that emerged from the eggs X 100

The total number of eggs laid.

Data Analysis

All data analyzed were computed using SPSS version 20.0.(SPSS Inc. Chicago, IL, USA) Standard deviation (SD) was generated using SPSS for mean values of the experiments to compare between means of egg viability and oviposition with effects of plant extracts treat with a control groups.

RESULT AND DISCUSSION

The result for the toxicity effect of plant extracts which are *Combretum micranthum, Xienmia americana* and *Aloysia citrodora* is presented in Table 1. The result showed that ethyl-acetate extracts of various concentration were potent on oviposition with *A. citrodora* causing low ovipostion with 42.85%. However, the ethanolic extract of *C. micranthum* also shows 8.84% when compare with control 90.00%.

The trend in the toxic effect was similar to methanol extract with 9.02% showing high significant when compare to control with a high effect o oviposition shown in Table 1. The result indicate significant difference ($p>0.05$) in the effects of plant extracts treated with different concentrations (ppm/mL) with the control. The result shown above, Table 2. Revealed the highly significant effect of *X. americana*

ethanol leaf extract at the concentration of 20.0 ml on the percentage of eggs viability, 10% when compared with control. Likewise at 20.0 ml and 30.0ml concentration, the ethanol and methanol extracts of *C. Micranthum* showed 40.0% and 26.0% effect on egg viability respectively (table 2).

While methanol extracts of *A. citrodora* shows low effects with 48.0% when compare with control. Table 2 shows significant (p>0.05) effect of *X. americana* ethanol leaf extract of at the concentration of 10 % on hatching ability was 26±06.21. Likewise at 20.0 ml and 30.0ml concentration the ethanol ad ethyl-acetate extract of *C. micranthum* and *A. citrodora* showed 40±01.70 and 40±04.6 hatching ability respectively while compared with control.

As plants are the natural factories of producing many phytochemicals having medicinal and insecticidal potential. Therefore, the present study is carried out to investigate the mosquito effect of the Indian medicinal plants *C. micranthum* and *Duranta plumieri* shows 20% effects against egg viability.

To study the mosquito effect with ethanolic extracts, various models were explored from the literature like field bioassay (Ansari *et al*., 2005) shows *Azadirachta indica* shows oviposition effects with17.5%, bioassay (Reagan et al. 2015), percentage and contact repellency bioassay experimental hut trial , egg viability bioassay method revealed methanol extracts has shown 78.0% effects against *Anopheles spp*.

In the present study, various extracts *X. americana* and *C. micranthum* has showed effect on egg hatching viability. While leaves ethyl-acetate extracts of *Duranta plumieri* and *Pogostemon benghalensis* were shows increasing order of polarity, ethanol and water o oviposition. The extracts were then screened for phytochemical tests using standard procedures to identify the effect on oviposition and egg viability against *Anopheles* mosquitoes. Another study, revealed ethanol and methanol extracts of *A. citrodora* were screened for tests using standard procedures to identify effects against *Anopheles* eggs and oviposit (Usman *et al.,* 2009).

Table 1: Effect of plant extracts on oviposition rate against female *Anopheles gambiae L.*

Solvents	Plant Material	Conc. (ppm)	No. of Mosquitoes tested	No. ± SE of Fully fed Mosquitoes	No. ± Mean of Gravid female Mosquitoes	Oviposition %
Ethanol	*X. americana*	100	100	41±16.00[a]	25±08.33[a]	12.07
		200	100	67±34.00[b]	33±11.00[b]	11.67
		300	100	44±02.00[c]	24±08.00[c]	09.27
		00	100	53±25.00[d]	28±09.33[d]	53.01
Methanol	*C. micranthum*	100	100	67±57.00[a]	10±03.33[a]	08.84
		200	100	29±06.00[b]	23±07.66[b]	46.07
		300	100	45±23.00[c]	22±07.33[c]	28.00
		00	100	80±65.00[d]	15±05.00[d]	80.00
Ethyl-acetate	*A. citrodora*	100	100	38±18.00[a]	20±06.67[a]	42.85
		200	100	40±08.00[b]	32±10.67[b]	40.74
		300	100	12±02.00[c]	10±03.33[c]	79.80
		00	100	95±61.00[d]	59±19.67[d]	90.00

SD= Standard Deviation; ±Mean; Concentration (ppm/mL); there is significant different (p>0.05), using one-way ANOVA

Table 2: Viability of *Anopheles gambiae L.* Eggs after treatment with plants extracts of different solvents

Solvent	Plant/Conc.(mg/ml)	No. of Eggs	No. ±S.D of Viable Eggs	Percentage (%) of Egg viability
Ethanol	*X. Americana*			
	10.0	50	26±06.21^{ab}	40.00
	20.0	50	10±02.65^{ac}	10.00
	30.0	50	19±07.42^{a}	28.00
	00.0	50	42±14.00^{ab}	66.00
	C. micranthum			
	10.0	50	10±00.92^{c}	20.00
	20.0	50	40±13.33^{ab}	22.00
	30.0	50	21±14.08^{a}	26.00
	00.0	50	36±12.00^{ab}	58.00
	A. citrodora			
	10.0	50	30±10.44^{a}	44.00
	20.0	50	28±09.15^{ac}	38.00
	30.0	50	40±04.62^{b}	24.00
	00.0	50	42±14.00^{c}	74.00
Methanol	*X. americana*			
	10.0	50	18±07.20^{ac}	28.00
	20.0	50	09±00.00^{bc}	09.00
	30.0	50	16±03.32^{b}	18.00
	00.0	50	41±06.81^{a}	82.00
	C. micranthum			
	10.0	50	19±08.10^{a}	26.00
	20.0	50	40±13.33^{b}	33.00
	30.0	50	16±10.08^{ab}	16.00
	00.0	50	36±12.00^{bc}	58.00
	A. citrodora			
	10.0	50	00±00.00^{a}	00.00
	20.0	50	00±00.00^{b}	00.00
	30.0	50	00±00.00^{a}	00.00
	00.0	50	25±08.33^{bc}	25.00
Ethyl-	*X. Americana*			
acetate	10.0	50	00±00.00^{a}	00.00
	20.0	50	00±00.00^{b}	00.00
	30.0	50	00±00.00^{c}	00.00
	00.0	50	91±30.00^{ac}	91.00
	C. micranthum			
	10.0	50	00±00.00^{a}	00.00
	20.0	50	00±00.00^{b}	00.00
	30.0	50	00±00.00^{c}	00.00
	00.0	50	60±24.50^{b}	60.00
	A. citrodora			

10.0	50	00±00.00[a]	00.00
20.0	50	00±00.00[b]	00.00
30.0	50	00±00.00[c]	00.00
00.0	50	45±30.31[ac]	35.00

SD= Standard Deviation; ±Mean; % =Percentage; Concentration (ppm/mL) (P>0.05), ANOVA. **Source:** Adopted from Ministry of Lands and Survey Makurdi (2015)

CONCLUSION

The three plant extracts exhibiting the maximum mosquitocidal activity which were selected for the study. Extracts were evaluated for oviposition and egg viability. Two were most stable for *Combretum micranthum* and *Aloysia citrodora*. The trend in the toxic effect of methanol extract shows high significance when compared to control with a high effect on oviposition. The result indicates a significant difference (p>0.05) in the effects of plant extracts treated with different concentrations (ppm/mL) with the control. Likewise, the concentration of ethanol and ethyl-acetate extracts of *C. micranthum* showed the effect on egg viability respectively. While the effect of *X. americana* ethanol leaf extract of at the concentration of 10 % on hatching ability. The concentration of ethanol and ethyl-acetate extract of *C. micranthum* and *A. citrodora* showed hatching ability respectively when compared with control. Hence the present research revealed that both ethanol and ethyl-acetate extract *C. micranthum* and acetone extract of *Aloysia citrodora* can be used as efficient, potent and safer mosquito control.

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