

Studies on the Potentials of Some Selected Plant Extracts Against Oocyst Formation in the Mid-Gut of Female *Anopheles gambiae* s. l.

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Abstract

Mosquitoes are of major public health concern since they are important disease vectors. This research aimed to evaluate the potential of some selected plant extracts using solvents of different polarity against oocyst formation in the mid gut of female *Anopheles gambiae* s.l. Plants materials were obtained from Goron-maje ward, Dambatta LGA, Kano State Nigeria. Healthy leaves of *Alyosia citrodora*, *Ximenia amaericana*, and *Combretum micrantum* were extracted using soxhlet and maceration techniques with ethanol, methanol and ethyl-acetate solvents. Mosquitoes were reared with the use of standard techniques. One hundred and fifty (150) female adults' mosquitoes were used

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and each was placed into four (4) different insectary containing different concentrations 100, 200 300 ppm with a control for each. Mosquitoes were dissected using standard procedure. The mid-gut was detached from thorax and then stained for oocyst count. Results shows that the oocyst density of female *An. gambiae* was lower with (5%) at 300 ppm of *X. Americana* for methanol, whereas ethanol and ethyl-acetate extracts of *C. micranthum*, *X. americana*, and *A. citrodora* inhibited low oocyst density by 10%, 5%, and 15% at 200 ppm using maceration method of extraction. The oocyst density seen was found to be lower at 300 ppm of *X. americana* using soxhlet extraction while ethanol and ethyl-acetate extracts of *C. micranthum*, *X. americana* and *A. citrodora* was found to inhibit lower oocyst density 10%, 5% and 15% at 200 ppm using maceration respectively. This study revealed that these plants extracts have remarkably effects in control of oocyst development in mid-gut of female *An. gambiae* s.l.

Key Words: Mosquito, Oocyst, Mid-gut, Plant Extracts

INTRODUCTION

Mosquitoes are of much concern to public health and well-being of the global human population (James, 2016). Since these mosquitoes transmit a number of dreadful diseases like filarial, malaria, elephantiasis and dengue, control measures using non-conventional insecticides like botanicals and phytochemical derivatives are gaining much attention in recent days due to a number of favorable reasons (Gericke, 2002). The impacts of phytochemicals on mosquito control strategies reveals some encouraging information on insecticides of plant origin have been tested to be specific in action against target insects and are non-toxic on the ecosystem and man as compared to the chemical compounds (Jeyabalan *et al.*, 2003).

One can speculate that people controlled and killed mosquitoes and other domestic insect pests by physically removing them or by using plant parts and plant derivative before the advent of synthetic chemicals. Larger number of human population in this world of about 80% depends largely on plants for health care control of infectious and non-infectious diseases (Werka *et al.*, 2007) Therefore, plants extracts are gaining acceptance in providing alternatives in vector control of diseases and arboviruses due to their non-toxicity, specificity, and are safe to the environment and ecosystem and have lesser effects to the flora and fauna (Ormancey *et al.*, 2000). However, these plants contain insecticidal phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects. Mosquitoes fed on human blood demonstrated higher feeding rates, fecundity, oviposition and fertility rates than those fed with other two (cattle and chicken) blood meal sources. Most of the earlier studies on mosquito rearing relied on human hosts or live animal hosts. (Boyd *et al.*, 2013) .

They transmit diseases as some pathogens complete their life cycles within the mosquito and they are passed onto humans during the time of biting (Tolle, 2009). Males do not suck blood but feed on plant juices (Foster and Walker, 2002). In Nigeria potential indigenous plants are highly recognized by herbalist as they employ widely for their bioactive chemical properties and vector control. Some of these locally used plant as alternative control of mosquitoes has not been prove on scientific fact for their effects against mosquitoes. *X. Americana*, *C. micranthum* and *A. citrodora* which were readily available in our locality and has been recognized by herbalist as source of mosquito control, yet have not been tested scientifically to ascertain their potential in the control of mosquitoes. The study aimed to

evaluate the potential of some plants extracted using solvents of different polarity against the density oocysts in the mid-gut of *An. gambiae*.

MATERIALS AND METHODS

Collection and Identification of *Anopheles gambiae* S.L:

Anopheles gambiae was obtained from the insectary unit, Department of Biochemistry, Bayero University Kano. Insect were authenticated by an expert in insect taxonomy at the Department of Zoology, Ahmadu Bello University, Zaria, Nigeria.

Collections and identifications of Plant Materials

Plants materials were obtained from Goron-maje ward, Dambatta LGA, Kano State. The plants were authenticated by an expert in plant taxonomy at the Department of Plant Biology, Bayero University Kano.

Processing of Plants Leaves

Healthy leaves of *A. citrodora*, *X. amaericana*, and *C. micrantum* were washed with tap water, cut into small pieces, air dried, and thereafter, they were ground into powder (Jeyabalan *et al.*, 2003).

Solvents

Ethanol, Methanol and Ethyl-acetate solvents were purchased from the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Bayero University Kano. The polarity of the solvents was confirmed by Azmir *et al.*, (2013).

Bioassay/Laboratory Investigation

To test the effect of plant leaf extract on the density of oocytes in the mid-gut of *An. gambiae*

To test the effect of plant extract on density of oocytes in the mid gut of *A. gambiae* the method described by Coleman, 2007 was adopted in which extracts. Extracts which were extracted using different solvent and methods were separately used. Three (3) day old starved female adult mosquitoes were feed with infected blood meal (gametocytes) consisting of plant extracts of different concentrations 100, 200, 300 ppm each, via feeding membrane method. One hundred and fifty (150) mosquitoes were assigned to three test groups. Mosquitoes were dissected

Dissection of Mosquitoes

Mosquitoes were dissected using procedure adopted by Coleman (2007). Few drops of phosphate buffer solution (PBS) was placed on a microscopic glass slide and mounted under the light microscope. Mosquito was anesthetized by stabbing the mosquito thorax with a need-tip probe. Mosquito legs were pulled off using finger or forceps and the mosquito back transferred onto the slide. The head was removed using forceps while holding down the mosquito thorax with the probe. Forceps were use to grasp the second to the last abdominal segment and the abdomen was pull off gently. The mid-gut was detached from the thorax. The mid gut was then stained and the oocyst were examined and counted Coleman (2007).

$$\text{Density of oocyst (\%)} = \frac{\text{No. of oocyst count}}{\text{No. of dissected mosquitoes tested}} \times 100$$

Data Analysis

Data analyzed were computed using version 20.0. (SPSS Inc. Chicago, IL, USA). Standard deviation was calculated based on the mean values of the experiments to compare between means of treatment and control groups. Comparison between means of treatment and control groups were used to perform analysis of variance (ANOVA).

RESULTS

The results show that soxhlet extraction of plant on the oocyst density of *An. gambiae* was lower (5%) at 300 ppm of *X. americana*, whereas ethanol and ethyl-acetate extracts of *C. micranthum*, *X. americana*, and *A. citrodora* inhibited low oocyst density by 10%, 5%, and 15% at 200 ppm using maceration (Fig. 1 and 2). However, the density of oocyst seen at the mid gut was found to be lower at 300 ppm of *X. americana* using soxhlet extraction while ethanol and ethyl-acetate extracts of *C. micranthum*, *X. americana* and *A. citrodora* was found to inhibit lower oocyst density 10%, 5% and 15% at 200 ppm using maceration respectively (Fig. 3).

DISCUSSION

The oocyst density seen at the mid-gut was found to be lower *A. citrodora* and *C. micranthum* with ethanol solvent using soxhlet extraction method. Histopathological changes of the mid-gut of *An. gambiae* treated with plant extracts at 100ppm and 300ppm using soxhlet extraction method shows the most damaging changes in the mid gut membrane when compared to control. This study recommends the possibility of using *Aloysia citrodora* and *X. americana* as bio-insecticide against *An. gambiae*. Activity of plant extracts, extracted solvent of different polarity using soxhlet and maceration method on oocyst formation in the mid-gut of *An. gambiae s.l.*. However, Earlier, Mulla and Su (1999) reported that these studies used different solvents such as ethyl acetate, ethanol and methanol to evaluate the effects of extract on transgenic plants activities against *An. gambia*. Similarly, Shalaby *et al.*, (1998) report on *X. americana* leaf extract has shown high effect of blocking the activity of oocyst development in the mid-gut with oocyst detected when compared to Generally, Shalaby *et al.*, (1998). Reported that further research should be done to confirm that the efficacy of botanical extracts against developmental of oocyst the mid gut of *An. gambiae*. This proved that these plants extracts has remarkably significant in controlling development of *An. gambiae s.l.* This research revealed that *Aloysia citrodora*, *C. micranthum* and *X. americana* extract can be used as efficient, potent and safer mosquito control.

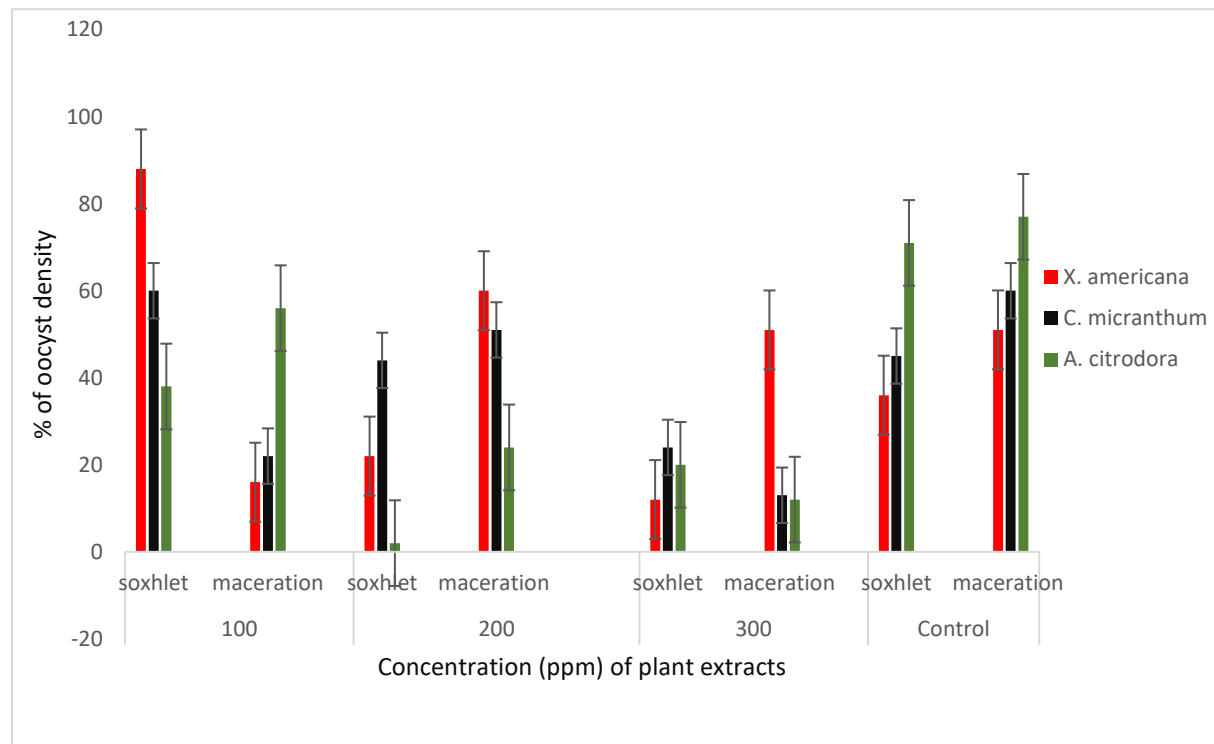


Fig 1: Activity of **methanol** plant extracts using soxhlet and maceration method on the (%) of oocyst in the mid-gut of *An. gambiae*

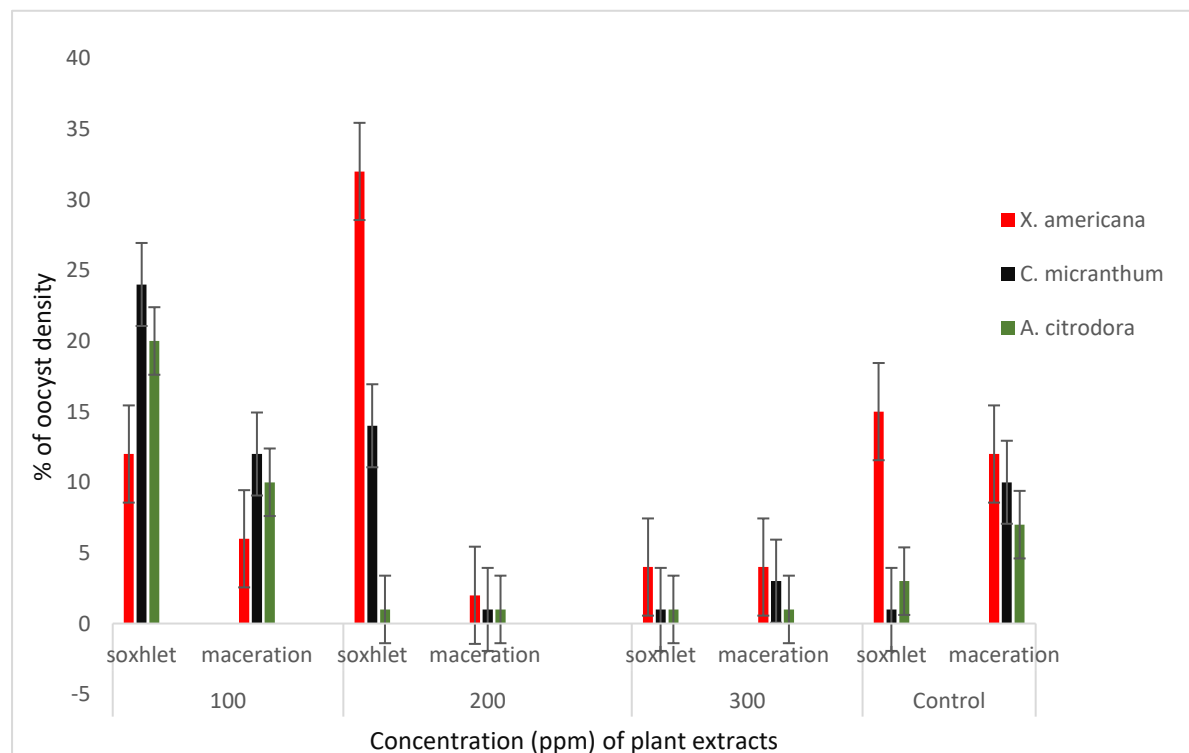


Fig 2: Activity of **ethanol** plant extracts using soxhlet and maceration method on the (%) of oocyst in the mid-gut of *An. gambiae*

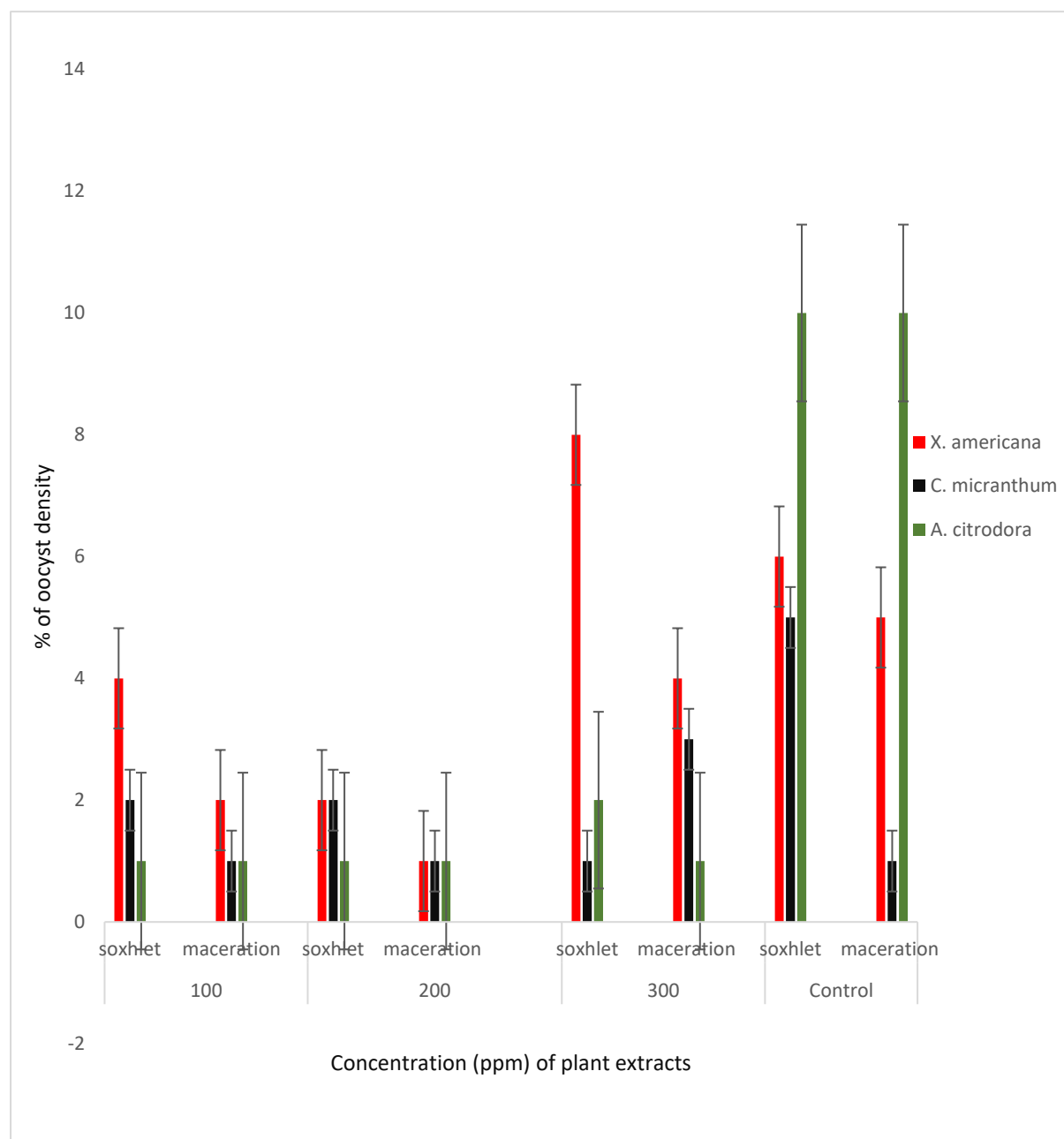


Fig 3: Activity of **ethyl-acetate** plant extracts using soxhlet and maceration method on the (%) of oocyst in the mid-gut of *An. gambiae*

CONCLUSION

The oocyst density seen at the mid-gut was found to be lower (5%) at 200 ppm and 300 ppm of *A. citrodora* and *C. micranthum* with ethanol solvent using soxhlet extraction method. changes of the mid-gut of *An. gambiae* treated with plant extracts at 100ppm and 300ppm using soxhlet extraction method shows the most damaging changes in the mid gut membrane when compare to control. This study recommends the possibility of using *Aloysia citrodora* and *X. americana* as bio-insecticide against *An. gambiae*. This proved that these plants extracts have remarkably significant in controlling development of oocyst in mid-gut of female *An. gambiae s.l.* with significant different compare to control at $P > 0.05$. This research

revealed that *Aloysia citrodora*, *C. micranthum* and *X. americana* extract can be used as efficient, potent and safer mosquito control.

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