Larvicidal Efficacy of *Vernonia amygdalina* and *Ocimum gratissimum* Extracts on Mosqutto Larvae

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ABSTRACT----
Background and objectives: Mosquito of the specie *Anopheles gambiae* is the carrier of most dreaded form of malaria known as falciparum malaria, caused by *Plasmodium falciparum*. This diseases has been recorded to kill hundreds to thousands of people yearly especially in Middle East and Africa. Controlling the vector is more preferable than treating the disease. This research is borne out of interest of low income earners or people living below standard securing their homes from these vectors. This research work focuses on monitoring the larvicidal efficacy of *Vernonia amygdalina* (common name-bitter leaf), *Ocimum gratissimum* (common name-scent leaf) extracts against mosquito larva (a stage in the life cycle of mosquitoes).
Methods: The plants for the research work were gotten from a location within Enugu metropolis, Nigeria. Their extractions were made using ethanol extraction method, phytochemical analysis of the extracts were carried out and the larvicidal efficacy of both (combined) extracts at different concentrations were tested on 25 mosquito larva.
Results: Of all the concentrations; 50mg/ml had the highest efficiency, destroying all the larva within 24hours of exposure, while the concentration of 2.5mg/mlrecorded the least mortality rate and having about six mosquito larva surviving even after 96hours of exposure.
Interpretation and conclusion: These plant extracts have proven to be effective at right concentration and therefore can be used as larvicides.

Keywords--- Extracts, mortality rate, mosquito larva, *Ocimum gratissimum*, phytochemical analysis, *Vernonia amygdalina*

1. INTRODUCTION

Mosquitoes transmit serious human diseases, causing millions of deaths every year (Kamaraj *et al*., 2011). *Anopheles gambiae* is the carrier of most dreaded form of the parasite; *Plasmodium falciparum*. Mosquitoes are among the well-known group of insect vectors that transmit deleterious human diseases, which pose as the major public health challenges affecting development in the poorest countries of the world (Awad and Shimaila, 2003). Thus one of the approaches for control of these mosquito-borne diseases is the interruption of the disease transmission, by destroying the mosquitoes or preventing mosquitoes from biting human being. The key to mosquito control is by larval control which can be done through modification of habitat with insecticides. Another way of controlling this infection is by minimizing the larval habitat especially in urban environment which include sealing of drains and soak ways, removing receptacles containing water such as old tins, tires etc. Where these physical measures are not possible, larvicides are usually applied. Larvicides are chemical substances or group of insecticides used to stop mosquito larvae from maturing into biting adults that cause diseases. The commonly and repeatedly used larvicides are fuel, oils, kerosene and insecticide formulations (Truman *et al*., 1976). The insecticides weaken the cuticle defense system of the larvae causing penetration of pathogenic organisms, thus reducing the mosquito population (Batabyal *etal*., 2007; Dua *et al*., 2009). Synthetic organic larvicides, although very efficacious to target species such as mosquitoes can be harmful to a variety of animal life including man. There is need for larvicides of natural origin for environmentally safe, biodegradable and target specific to combat mosquito species as vectors of some human deleterious diseases (malaria, filariasis, encephalitis. etc.) which have caused a great threat to human existence at the larval stage. Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases (Mark and John, 2002). Mosquitoes transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. Mosquitoes pass through four distinct stages in their life cycle: egg, larva, pupa and adult. Prodigious numbers of mosquitoes can hatch simultaneously under the proper conditions. The mosquito larva has a well-developed head with mouth brushes used for feeding, a large thorax with no legs, and a segmented abdomen. The larvae of all mosquitoes live in still, non-flowing water. Some species live in permanent swamps and marshes, some in water contained in tree holes or other containers (tires, catch basins, etc.), and some

develop in areas that are only wet for a period of time (such as vernal pools). Bitter leaf, isa member of the *Asteraceae* family, genus *Vernonia*. Its scientific name is "*Vernonia amygdalina".* The leaves are very economic and are up to 20 cm long.*V. amygdalina* is commonly called bitter leaf in English because of its bitter taste. The Igbo speaking people in Nigeria use the bitter leaf mostly as vegetable when washed to prepare soup while the Yorubas (Nigeria) use it more as medicine. The leaves are also taken as an appetizer and a digestive tonic by some people. Bitter leaves are widely used for fevers and medicinal concoction; usually taken as laxative among local people.The scent leaf (common name) has the botanical name- "*Ocimum grattissimum*", it belongs to the Family "*Labiatae*". It is commonly grown around houses as a mosquito repellant. The leaf serves as a decongestant for head colds, bronchitis and sinusitis. The leaf is also chewed traditionally for all tooth and gum disorders and it also has various other medicinal usages. These two plants mentioned are common plants grown in various homes within Nigeria and still very much affordable at markets, because of this they lie waste in several homes. Therefore this research work focuses on other economic use of these plants for other functions apart from home consumption and the uses listed above. This research work is borne out of interest to check for the plants' efficacy against mosquito larva and if recorded positive, homes and producers of larvicides will be encouraged to make use of these plants in controlling mosquito larva breeding in stagnant water.

2. MATERIALS AND METHODS

Plant Collection and Identification: FreshScentand Bitter leaves were collected from Akama Oghe in Ezeagu Local Government Area, Enugu State, Nigeria. They were identified by a botanist as *Ocimum gratissimum* and *Vernonia amygdalina* respectively.

Extraction of Crude Extract using ethanol: The leaves were washed in tap water, shade-dried for five days and were ground into fine powder using electric blender. Ethanol extract of the leaves *Vernonia amygdalina* and *Ocimum gratissimum* were made by weighing out 150g of the powdered leaves separately. Each of the weighed leaf was soaked in 5litres plastic gallon containing 2500ml of ethanol, this was properly kept homogenous by constant shaking for 72 hours. It was sieved with muslin cloth, the filtrate was filtered again using Whatmans' filter paper No.1. The final filtrate of each leaf (inside different containers) was allowed to evaporate for 4 days in order to obtain the residue (the crude extract), these were stored for further research work.

Preparation of Stock Solutions: Stock solutions were prepared by dissolving 5g of each crude extract in 150mls of distilled water and 1 drop of acetone was used to emulsify the oils in extract. All the test solutions were made by pipetting out 2.5mg/ml, 12.5mg/ml, 25mg/ml, 37.5mg/ml, and 50.0mg/ml of the stock solutions into 250ml of water in separate labeled white plastic bowls.

Rearing of mosquito larva:The Mosquito larva were collected from water receptacles like guttersby placing buckets with water and decayed dried fish in a gutter supposed to harbor mosquito larva, also earthen pots were purposefully left outside as traps for these insects. The receptacles were left outside for 5days for proper trapping of the vector eggs and larva. After hatching, first instar larva were distributed in bowls 30cm in diameter and 12.5m in depth. Care was taken to prevent overcrowding until development to early 4th instar larvae required for the study was achieved. The larva were kept in the plastic buckets half filled with tap water and fed with 2g of Quaker oat every 24 hrs. Water in rearing container was changed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface or the subsequent larva stages. This technique was repeated several times to provide the needed instar for the larvicidal test.

Larvicidal assay: The bioassay was performed at a room temperature of $27^oC \pm 10^oC$, Relative humidity 70-85%, Photoperiod 14:0 (light: dark) and pH 7.0 of distilled water. Twenty five (25) mosquito larvawere selected by means of rubber pipette andwere placed in five small specimen bottles containing 25ml distilled water, these were exposed to each of the concentrations of the extracts in a final volume of 245ml distilled water taken into white plastic bowls. The larvae in all the bowls were fed every twenty four hours with the same quantity of Quaker oat powder (2g) which was spread evenly across the water surface. Five replicates of the test concentration and control (without the plant extracts) were tested for anti-larval effects. The larval mortalities were recorded at intervals of 24, 48, 72 and 96 hours of exposure and the percentage mortality was recorded.

Phytochemical analysis: Phytochemical analysis is a test carried out on sample extracts to test for the presence of the following active components; Alkaloids, Flavonoids, Tannins, Saponins, Phenol, Steroids, and Glycosides.

Test for alkaloids: 1ml of 1% HCL was added to 3ml of each of the extract in a test tube. The Mixture was heated for 20minutes in a water bath. It was allowed to cool and filtered. 1 ml of each filtrate was added 0.5ml of Mayer's reagent and was then observed for creamy color change. Froth and Emulsion test were also carried out.

Test for Saponins

a) **Frothing test:** 3ml of each extract was diluted with 2ml of distilled water in a test tube, the mixture was shaken vigorously. A persistent frothing movement was observed for positivity.

b) Emulsion test: 3ml of each extract was added to 5 drops of olive oil in a test tube and the content was vigorously shaken. Emulsification signifies positive.

Test for flavonoids: 3ml of each extract was added to 10ml of distilled water, the solution was shaken properly and 1ml of 10% NaOH solution was added to the mixture. And yellow coloration was observed.

Test for steroids:

a) Salkowski test: 5 drops of conc. H_2SO_4 was added to 1ml of each extract in a separate test tube. A red coloration was observed and noted.

Test for tannins: 2ml of each extract was put in a separate test tube, boiled gently for 2 minutes and was allowed to cool. Then 3 drops of ferric chloride solution was added to each.

Green coloration was observed.

Test for phenols: 1ml of dissolved extract was added to 1ml of distilled water and few drops of 5% NaOH. Orange coloration observed.

Test for glycosides: 1 ml of each extract was pipette into a test tube, and 10ml of 50% H_2SO_4 was addedto the test tube, the test tube was heated for 15 minutes and allowed to cool. 10ml of Fehling solution was added to the above mixture and was boiled again.Brick red ppt. was observed.

3. RESULTS

The effects of the two plants (*Ocimum gratissimum* and *Vernonia amygdalina)* against the larvae of mosquito after 24, 48, 72, and 96 hours of exposure are shown in Table 1 below. There was100% mortality of the mosquito larvae within 24hrs of exposure in SE (sample E) containing 50mg/ml concentration of the extracts. SD (Sample D) containing 37.5mg/ml concentration of the extract produced 100% mortality against the larvae at 48 hours of exposure. Sample C, after 72 hours of exposure showed moderate level of toxicity against the larvae at concentration of 25 mg/ml. Sample B with concentration of 12.5 mg/ml and 72 hours of exposure had 100% mortality, but with the lowest mortality rate and concentration. Sample A with concentration of 2.5 mg/ml showed very low level of toxicity to the larvae after 92 hours of exposure. The control which contained only the mosquito larvae in distilled water and Quaker oat with no extract,showed no toxicity. The percentage mortality rate of the larva at different concentrations is shown in figure 1.
The result of the phytochemical analysis test which was carried out in order to check for the presence of the active ingredients such as; Alkaloids, Flavonoids, Tannins, Saponins, Phenol, Steroids, and Glycosides in the two plant extracts and this result is shown in Table 2 and Table 3 for *Ocimum gratissimum* and *Vernonia amygdalina*respectively.

Table 1: Mortality rate of the mosquito larva at different concentrations of the plant extracts

Plant Extract	Concentration (mg/ml)	Number of Mosquito larva used	Mortality rate at 24hrs	Mortality rate at 48hrs	Mortality rate at 72hrs	Mortality rate at 96hrs
SA	2.5	25	6	6	6	6
SB	12.5	25	13	18	25	25
SC	25.0	25	17	25	25	25
SD	37.5	25	20	25	25	25
SE	50.0	25	25	25	25	25
Control	0	25	0	0	0	0

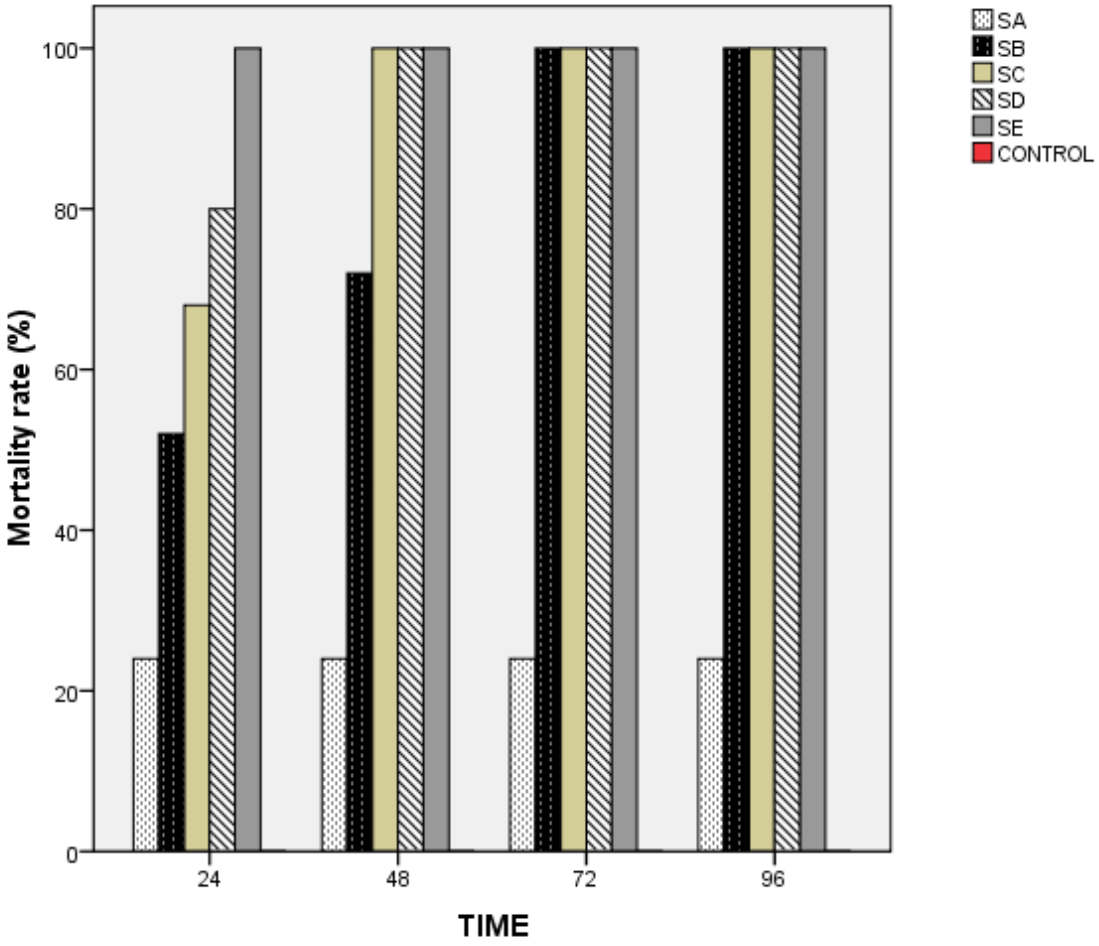

Fig. 1: Percentage mortality rate of mosquito larvae at different concentrations of the plant extracts.

Table 2: Phytochemical Composition of *O. gratissimum* extract

Phytochemical parameters	**Status**
Alkaloid	+
Saponins	++
Tannin	++
Flavonoid	+
Steroid	-
Phenol	-
Glycoside	-

Key: + = Positive, ++ =highly positive, - = negative

Table 3: Phytochemical Composition of *V. amygdalina* extract

Phytochemical composition	Status
Alkaloid	++
Saponins	++
Tannin	++
Flavonoid	++
Phenol	-
Steroid	-
Glycoside	-

Key: + = Positive, ++ =highly positive, **-** = negative

4. DISCUSSION

This study was carried out to examine the larvicidal efficacy of the two plant leaves; *Vernonia amygdalina and Ocimum gratissimum* against the larvae stage of mosquito. The results are represented in table 1 and 2 above. The two plant extracts exhibited good larvicidal activities on the mosquito larvae with varying susceptibility. The varying susceptibility observed here was in line with reports from previous findings that various mosquito species showed differential susceptibility to different plant extracts Pathak *et al.*, (2000). Sample E exhibited the highest mortality rate after 24hr of exposure at the concentration of 50mg/ml by eliminating all the larvae at once. Sample D had mortality rate which was close to the highest concentration at concentration of 37.5mg/ml. The sample C was able to destroy seventeen (17) mosquito larvae at concentration of 25mg/ml after 24hours.

Sample B ranked next to the least concentration and was able to have attained the LC_{50} since it was found to kill up to thirteen (13) mosquito larvae after 24hours of time of exposure at concentration of 12.5mg/ml. While sample A after 24hours destroyed only six (6) mosquito larvae at concentration of 2.5mg/ml. Other samples apart from sample E completed their mortality rates at later hours of 48hours, 72hours and 96hours. Average mortality indicate that theextracts exhibited significant mortality rate on the targeted mosquito larvae. The bioassay showed that the toxic effect of the extracts were proportional to the concentrations with the highest concentration being the most effective. Figure 1, showed the Percentage mortality rate of mosquito larvae treated with different concentrations of the plant extracts. This simply means that at concentration of 50mg/ml for 24hours, there was 100% mortality rate. Whereas the lowest concentration at 2.5mg/ml yielded only 24% mortality rate throughout the different hours of exposure. This is in support of the findings ofOkigbo*et al.*,(2010). Table 2 and 3 shows the phytochemical analysis of*Ocimum gratissimum* and *Vernonia amygdalina* respectively. The phytochemical composition of the two extracts indicates that *V.amygdalina* has more of the Alkaloids and Flavonoids than *O.gratissimum,* whileother components like Steroids, Phenol, and glycosides were completely absent in both extracts. The four phytochemical components are responsible the plants' mortality rate on the mosquito larvae, but *V. amygdalina* has more efficacy based on the result.Okigbo*et al.*,(2010),discovered that *O.gratissimum* is more effective when combined with *V.amygdalina*or any other medicinal plant, and this is proven in this particular work. Also, the research work carried out by Adeniyi *et al*., (2010)suggested that *Vernonia amygdalina* leaf has a high significant mortality rate. Figure 3, expressed the Percentage mortality rate of the mosquito larvae with time.

5. CONCLUSION

This study has proven that the extracts of*Ocimum gratissimum* and *Vernonia amygdalina* possess chemical compounds that are active against mosquito larvae and is believed to be a promising strategy in controlling mosquito population in our society. Therefore these plant extracts could be formulated into mosquito larvicides to prevent mosquito borne diseases. Larvicides are tremendous effective weapons in the fight against insects carrying diseases, of which these extracts are among the larvicides. Therefore, individuals are encouraged to pour the liquid extract of these plants into any stagnant water around homes to control mosquito and in turn controlling malaria which is a deadly infection, killing all ages.

6. REFERENCES

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