LABORATORY EVALUATION OF ETHANOLIC AND METHANOLIC EXTRACTS OF *OCIMUM GRATISSIMUM* AGAINST LARVA OF *ANOPHELES GAMBIAE* AND NON-TARGET ORGANISMS

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ABSTRACT: Methanolic and ethanolic extracts of *Ocimum gratissimum* leaves were evaluated for larvicidal activity against larva of *Anopheles gambiae* and non target organisms in the laboratory. Photochemical analysis of leaf showed presence of tannins, alkaloids, flavonoid, cyanogenic glycosides, cardiac glycosides and saponin. The organisms were exposed to different test concentrations ranging from 20mg/ml to 100mg/ml for 96hours. Larvae of *A. gambiae* were susceptible to the extracts. Ethanolic extracts of the leaf was more active with LC$_{50}$ and LC$_{90}$ of 60.9mg/ml and 464.4 mg/ml respectively while the LC$_{50}$ and LC$_{90}$ of methanolic extracts of leaf were 73.6mg/ml and 1021mg/ml respectively. The extracts exhibited insect growth regulating activity and were found to be safe to non target organisms. Results showed that larvicidal effect of the methanolic extract can be compared to that of neem leaf extracts.

KEY WORDS: *Ocimum gratissimum* leaves, methanolic and ethanolic extract, larvicidal activity, *Anopheles gambiae*, non target organisms.

Mosquitoes are important vectors of diseases and nuisance pests (Azhari et al., 2009). Mosquito-borne diseases remain a major problem in the world particularly in tropical and subtropical regions (Bernard, 1999). Over two million people are at risk of diseases caused by mosquitoes (Odalo et al., 2005). Repeated use of synthetic insecticides for mosquito control has resulted into development of resistance in many vector species, undesirable effects on non-target organisms, environmental and human health concerns (Das et al., 2007). These problems coupled with high cost of the insecticides have revived interest in exploiting the pest control potentials of plants (Grainge & Ahmed, 1988). Despite their application as general toxicants against immature stages of mosquito, their phytochemicals may have potential uses as growth and reproduction inhibitors, repellants and oviposition deterrents (Savignaname & Kalyanasundaram, 2004).

Many species of *Ocimum* belonging to the Family Lamiaceae and Order Lamiales (USDA, 2008) a perennial scented shrub with lime fizzy (Wagner et al., 1999) have been reported to have larvicidal activity, *O. basilicum* (Azhari et al., 2009) *O. sanctum* (Vinayagam et al., 2008) and *O. americana* (Cavalcanti et al., 2004). Oil extracts of *O. gratissimum* leaves have been reported to have repellant effect on adult mosquitoes (Oparaocha, 2008). Traditionally, its fresh leaves are placed on a lighted lamp and the fumes have been reported to repel adult mosquitoes from target organisms. *Ocimum gratissimum* have been reportedly
used in the treatment of fever and diarrhea (Oliver, 1980), convulsion, stomach
pains and catarrh (Oparaocha, 2008) among many other uses. However, there is
still dearth of information on its effects on immature stages of mosquitoes. This
study examines the effects of *O. gratissimum* on the larvae of *Anopheles gambiae*
and other non-target organisms.

**MATERIAL AND METHODS**

**Preparation of stock solution of plant leaf extract**

Fully developed leaves of *O. gratissimum* were collected in the month of June,
2011. The leaves were thoroughly washed, shade dried and ground with the
warring blender. Ground leaves were sieved to get fine powder. Methanolic and
ethanolic extracts were obtained by soaking 100gm of finely ground plant
material in 500ml of 70% methanol and ethanol respectively for 24hr with
periodic shaking. The content of each set up was filtered using Whatmann’s No 1
filter paper and the residue evaporated to dryness using the soxhlet apparatus.
After this, the extract was allowed to cool and then each... was put into a container
and labeled accordingly. 20mg, 40mg, 60mg, 80mg and 100mg of each of these
were mixed with 100ml of ethanol or methanol as the case may be to get test
centrations to be used.

**Effects of plant leaf extract on mosquito larva**

Ten active larva were put into 500ml transparent containers which have
249ml of dechlorinated water, fed with glucose and allowed to acclimatize. 1ml of
each test concentration was added to each container and labeled accordingly. Two
replicates for each test concentration ranging from 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, 100mg/ml and a control( made up of 249ml of
dehlorinated water and 1ml of ethanol(or methanol) were setup and observed
under room temperature of 30°C ± 2°C.

Mortality of larvae were observed at 6hr intervals for 96hr. the larvae were
also observed for effect of extract on their growth, other abnormalities like
sluggishness and reduced swimming activity in each container of the different test
centration for 96hrs.

**Effect on non-target organisms**

The experimental set-up for the larvae was also used against non-target
organisms. The organisms used were water skater, water strider (*Alierris remigis*)
and tadpole of toad (*Bufo regularis*). One organism per container was used to
avoid predation and each set-up observed for mortality and other abnormalities
like reduced swimming activity, sluggishness etc for 24hrs.

**Phytochemical screening of plant leaf material**

Phytochemical screening of leaf material for saponins, tannins, flavonoids,
alkaloids, cynogenic and cardiac glycosides was carried out according to Amadi et
al. (2004).

**RESULTS**

Methanolic and ethanolic extracts of leaves of *O. gratissimum* showed some
larvicidal activity on *A. gambiae* tested at concentrations ranging from 40mg/ml
to 100mg/ml. No mortality was recorded for each at test concentrations less than
40mg/ml. The results are as shown in tables 1 and 2.
Ethanolic extract of leaf material was more effective than methanolic extract of leaf material of *O. gratissimum* as shown in table 3.

It was observed that the leaf extracts tested on the mosquito larva affected the growth rate and swimming activities negatively. Sluggishness and other morphological abnormalities were observed. The time onset of observed behavioral changes reduced with increasing extract concentration (table 4).

The effect of the plant extract increased with increasing test concentration. No abnormal behavior was observed in the control. Pupal emergence occurred after 4 days in the control while it occurred after 5 days in the 20 mg/ml test concentration experiment and there were no other observed effects. Pupal emergence in the 40 mg/ml assay emerged 6 days for ethanol extract and 7 days in methanol but the emerged pupa were not as active as those emerging in the control, they could not molt to the imago stage but died later on. Those that survived in 60 mg/ml, 80 mg/ml and 100 mg/ml (methanol) after 96 hr could not molt to pupa but also die later on.

Phytochemical screening result revealed the presence of tannins, saponins, alkaloid, flavonoid, cyanogenic and cardiac glycosides in varying concentrations / ranges (table 5).

Saponins occurred in trace amounts while flavonoids concentrations were high.

There were no observed adverse effects on non-target organisms. No abnormality or mortality was observed on the non-target organisms after 24 hr exposure to different test concentrations of both methanolic and ethanolic extracts of *O. gratissimum* leaf.

**DISCUSSION**

The study has shown that methanolic and ethanolic extracts of *O. gratissimum* leaf have growth and larvicidal effects at high concentrations have growth regulatory and larvicidal effect on *Anopheles gambiae*. The result has shown that *O. gratissimum* leaf extracts have larvicidal effects on *A. gambiae* with 100% mortality at 100 mg/ml of leaf extract. Larvicidal effects of other species of *Ocimum* such as *O. basilicum* on *Anopheles gambiae* (Azhari et al., 2009), *O. sanctum* on *A. stephensi* (Vinayagam et al., 2008) and *O. americana* on *Aedes aegypti* (Cavalcanti et al., 2004) have been reported. Mortality could not be linked to the solvents ethanol and methanol as no death was recorded in the control containers. The Larvicidal effect could be linked to phytochemicals present in the leaf. The presence of alkaloids in plants may be associated to their biological activities (Basu & Basa, 1972). Crude extracts of saporium from fruit pods of *Sivartzia madagascarinsis* produced high mortality in *Anopheles gambiae* larvae (Minija & Sarda, 1986).

The phytochemicals may also be responsible for the growth abnormalities and delay in the pupal emergence observed in the course of the study. Sivagnaname & Kalyanasundariam (2004) and Mohtar et al., (1999) reported prolonged pre-imago period, for *Aedes aegypti* larva exposed to methanol extract of *Nerium indicum*. The test concentrations recorded larvicidal activities in concentrations from 40 mg/ml and above while concentrations below 40 mg/ml were sub lethal. The LC50 and LC90 for ethanolic extracts were 60.9 mg/ml and 464.1 mg/ml respectively while those for methanol extracts were 73.6 mg/ml for LC50 and 1021 mg/ml for LC90. Some plant extracts have been shown to exhibit larvicidal activity or insect growth regulatory activity against mosquito larvae at concentration above 10 mg/ml (Deshmukh & Renapurka, 1987; Thangam&
Kathiresan, 1988). Sivagnaname & Kalyanasundaran (2004) reported LC$_{50}$ values less than 0.2mg/ml for methanolic extracts of *Atlantia monophylla* against larvae and pupae of *Anopheles aegypti* and *Culex quinquefasciatus*. Nevertheless the LC$_{50}$ of 60.9mg/ml of ethanolic extracts of *O. gratissimum* can be compared to LC$_{50}$ of 55-65mg/ml for some Neem extracts as reported by Ascher & Meisner (1989).

**CONCLUSION**

Ethanolic extracts may be superior to methanolic extracts considering their LC$_{50}$ and LC$_{90}$ values. It could be that ethanol as a solvent extracted more of the phytochemical components than the methanol. It could also be that the more the concentration of leaf materials the more the larvicidal and growth inhibitory activities of the leaf extract. The result revealed that the leaf extract was safe for non-target organisms as no adverse effects or mortality was recorded. This shows that the extract if used in the field where these organisms co-inhabit with the mosquito larvae will only kill the larvae and not harm other organisms.

Resistance of mosquito vectors to synthetic insecticides, high cost of these insecticides and their adverse effects on non-target organisms and environment has been a major concern in vector control. Botanical insecticides are reported to be effective against insect pests, eco-friendly with reduced effect on non-target organisms, cheap, readily available, biodegradable and safe, they may serve as alternative to synthetic ones (Sivagnaname & Kalyanasundaram, 2004; Dehghan et al., 2012; Mufutau, 2012).

**LITERATURE CITED**


Table 1. Mortality rate of Ethanolic leaf extract of *O. gratissimum* on larvae of *A. gambiae*.

<table>
<thead>
<tr>
<th>Concentration mg/ml</th>
<th>Total no</th>
<th>No dead</th>
<th>% mortality</th>
<th>Log10 concentration</th>
<th>Probit</th>
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<tbody>
<tr>
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<td>2.0</td>
<td>8.09</td>
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</table>

Table 2. Mortality rate of Ethanolic leaf extract of *O. gratissimum* on larvae of *A. gambiae*.

<table>
<thead>
<tr>
<th>Concentration mg/ml</th>
<th>Total no</th>
<th>No dead</th>
<th>% mortality</th>
<th>Log10 concentration</th>
<th>Probit</th>
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<td>8</td>
<td>80</td>
<td>2.0</td>
<td>5.84</td>
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</tbody>
</table>

NB: Total number and number dead were average of the two replicates for each test concentration.
Table 3. LC$_{50}$ and LC$_{90}$ values of methanolic and ethanolic leaf extracts.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Y</th>
<th>R$^2$</th>
<th>LC$_{50}$</th>
<th>LC$_{90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Y=3.502x – 1.538</td>
<td>0.893</td>
<td>73.6mg/ml</td>
<td>1021mg/ml</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Y=4.534x – 3.092</td>
<td>0.998</td>
<td>60.9mg/ml</td>
<td>464.4mg/ml</td>
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</tbody>
</table>

Table 4. Observation time of the abnormalities in *A. gambiae* larvae in different test extract concentrations.

<table>
<thead>
<tr>
<th>Test concentration (mg/ml)</th>
<th>Time of observation (Hours)</th>
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<tbody>
<tr>
<td></td>
<td>Methanol</td>
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<tr>
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<td>36</td>
</tr>
<tr>
<td>100.0</td>
<td>18</td>
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</table>

Table 5. Presence of Photochemical in the leaf extract of *O. gratissimum*.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>++++</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+++</td>
</tr>
<tr>
<td>Cyanogenic glycoside</td>
<td>+++</td>
</tr>
<tr>
<td>Tannin</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>++</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
</tbody>
</table>

- Absence, ++++ High concentration, +++ & ++ Moderate concentration, + Trace concentration.