Larvicidal activity of *Ocimum gratissimum* and *Solenostemon monostachyus* leaves on *Anopheles gambiae*

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This study presents larvicidal activity of *Ocimum gratissimum* and *Solenostemon monostachyus* leaves extracts on *Anopheles gambiae*. Leaves contain alkaloids, saponins, phenolics, tannins, essential oils, lectins, glycosides and phytic acid. Larvicidal activity was determined by subjecting *Anopheles gambiae* larvae to different concentrations (12.5, 23.50, 100 and 200 ppm) of acetone and aqueous extracts of leaves using *in vitro* method at room temperature for 72 h exposure. Mortalities were respectively 85% and 45% for acetone and aqueous leaves extracts of *O. gratissimum* and 100% and 50% for acetone and aqueous leaves extracts of *S. monostachyus*. LC50 were respectively 52 ppm and 232 ppm for acetone and aqueous leaves extracts of *O. gratissimum* and 28 ppm and 200 ppm for acetone and aqueous leaves extracts of *S. monostachyus*. Leaves of *S. monostachyus* exhibited higher toxicity than leaves of *O. gratissimum*. Acetone extracts were more potent than aqueous extracts and tested significant at P< 0.05. Lethal activities were found to be dose dependent.

Keywords: *Anopheles gambiae* larvae, Larvicidal activity, Leaves, *Ocimum gratissimum*, *Solenostemon monostachyus*

Introduction

Out of 70 species of *Anopheles* that transmit malaria1, only 40 are of major significance. *Anopheles* mosquitoes are mostly frequently found in tropical region, but are also found in temperate climates and in the arctic region during summer. Since mosquitoes can have many generations per year, high levels of resistance can arise very quickly2. Over 125 species of *Anopheles* mosquitoes3 have developed resistance to insecticides used for indoor residual spraying. Some important measures in mosquito control2 are discouraging egg laying, preventing development of eggs into larvae, killing adult mosquitoes and preventing mosquitoes’ bite. Rapid increase in resistance to synthetic insecticides is a threat to public health. Insecticide of biological origin may serve as suitable alternative biocontrol technique to eradicate both resistance and non- resistance insecticide based- *Anopheles* mosquito.

This study determines larvicidal activity of *Ocimum gratissimum* and *Solenostemon monostachyus* leaves extracts on *A. gambiae*.

Experimental Section

Preparation of Leaf Extracts

Fresh leaves of *S. monostachyus* and *O. gratissimum*, collected at Umuogba, Abia State, Nigeria, were washed with fresh sterilized water, dried under shade at room temperature (RT) for 14 days and pulverized using electronic grinder. Leaves powder (40 g each) was extracted by maceration in 400 ml each of acetone and water for 72 h. Extracts were filtered, evaporated to dryness at RT in a steady air current4. Concentrations of extracts were determined by evaporating extracts (0.1 ml each) in an evaporating dish of known weight in an oven to dryness. Dish containing residue was allowed to cool and weighed. Residue weight was obtained by subtracting weight of empty dish from the weight of dish and residue. This process was repeated in triplicate in each case. Phytochemical analysis of leaf extracts was done by using reported method5. For antibacterial activities, 512 mg.ml of each extract were used for screening, after dissolving 2.1 g of extracts in 4 ml of each of extracting solvents6.

Mosquito Larvicidal Assay

Mosquito larvae of *A. gambiae*, collected from High TECH parasitology laboratory at Port-Harcourt, River
State, Nigeria, were allowed to emerge in plastic containers filled with water. At II instars stage, larvae were transferred to large buckets (37 cm x 31 cm x 6 cm), fed on Tetramin fish food at 1 mg per beaker per day and water temperature was maintained at RT (28±2°C) throughout larval development. Larvicidal assay was carried out by exposing 20 early IV instar larvae of *A. gambiae* to various concentrations of acetone and aqueous extracts. Different concentrations (200, 100, 50, 25 and 12.5 ppm) of extracts were added in beakers containing larvae in 100 ml of water-sample solution and 0.1 ml acetone. Control experiment contained only 0.1 ml of acetone (blank). Test was triplicated from separately reared batches of larvae. Numbers of dead larvae were taken per 24 h for 3 days.

**Statistical Analysis**

Average mortality data obtained from larvicidal activity were subjected to probit analysis for calculating LC50, LC90, statistical slope and chi square values by adopting reported method. Results greater than tabular values at P ≤ 0.05 and P ≤ 0.01 were considered to be statistical significant.

**Results**

Phytochemical analysis of leaf extracts of *O. gratissimum* and *S. monostachyus* respectively, gave: alkaloids, 22.76, 8.13 mg/100g; saponins, 3.09, 8.61 mg/100g; tannins, 8.03, 7.41 mg/100g; phenolics, 2.13, 1.25%; essential oils, 0.09, 0.08%; phytates, 17.54, 15.59%; cardiac glycosides, 3.16, 2.76 mg/ml; and lectins (Hemag unit) 46, 16 mg. *O. gratissimum* contained more of alkaloid, tannin, phenolics, essential oil, phytate, glycosides and lectin compared to *S. monostachyus* that had only more of saponin. Larvicidal bioassay of different concentrations of acetone extract of leaves (Table 1), after 72 h of exposure of 20 early IV instar larvae of *A. gambiae*, showed mortality maximum (100%) for *S. monostachyus* at 200 ppm and 45% at 12.5 ppm, whereas for *O. gratissimum*, mortality were maximum (85%) at 200 ppm and 40% at 12.5 ppm. Larvicidal bioassay of different concentrations of aqueous extracts of leaves (Table 1), after 72 h of exposure of 20 early IV instar larvae of *A. gambiae*, indicated that *S. monostachyus* showed greater larvicidal activity than *O. gratissimum*. No mortality was recorded in control set-up using 0.1% acetone and 0.1% distilled water. Probit analysis of larvicidal bioassay of different extracts of leaves (Table 2) in a 72 h experiment showed LC50, LC90, slope, DF and X2 value of respective extracts, and indicated that highest mortality was recorded in acetone extracts. Acetone extracts of *S. monostachyus* (ASM) required lowest concentration of extracts to exert larvicidal activity compared to acetone extracts of *O. gratissimum* (AOG). *S. monostachyus* showed greater larvicidal activity than *O. gratissimum*. When mortality

### Table 1 – Mortality of *Anopheles gambiae* larvae using leaf extracts [control: acetone (0.1%); distilled water (0.1%)]

<table>
<thead>
<tr>
<th>Conc., ppm</th>
<th>Mortality (%) in acetone extracts</th>
<th>Mortality (%) in aqueous extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>O. gratissimum</em></td>
<td><em>S. monostachyus</em></td>
</tr>
<tr>
<td>200</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>75</td>
<td>85</td>
</tr>
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<td>50</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>25</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>12.5</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>control</td>
<td>-</td>
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</table>

### Table 2 – Larvicidal bioassay of different extracts of leaves

<table>
<thead>
<tr>
<th>Extracts</th>
<th>LC50, ppm</th>
<th>LC90, ppm</th>
<th>Slope</th>
<th>DF</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASM</td>
<td>28.00</td>
<td>164.00</td>
<td>17.67</td>
<td>4</td>
<td>20.00</td>
</tr>
<tr>
<td>WSM</td>
<td>200.00</td>
<td>468.00</td>
<td>28.44</td>
<td>4</td>
<td>7.06</td>
</tr>
<tr>
<td>AOG</td>
<td>52.00</td>
<td>216.00</td>
<td>18.91</td>
<td>4</td>
<td>10.55</td>
</tr>
<tr>
<td>WOG</td>
<td>232.00</td>
<td>508.00</td>
<td>31.00</td>
<td>4</td>
<td>4.78</td>
</tr>
</tbody>
</table>

ASM, acetone extract of *S. monostachyus*; WSM, water (aqueous) extract of *S. monostachyus*; AOG, acetone extract of *O. gratissimum*; WOG, water (aqueous) extract of *O. gratissimum*; Control, nil mortality; Significant at P<0.05 level; LC50, lethal conc. that kills 50% larvae; LC90, lethal conc. that kills 90% larvae; DF, degrees of freedom; X², Chi square
of two solvent extracts were analyzed using chi square, ASM and AOG were tested significant at 95% confidence level (P ≤ 0.05).

Discussion

Phytochemicals present in various extracts were responsible for larvicidal activities\(^9\)\(^-\)\(^11\) of leaf extracts, since extracting solvents gave nil mortality. Extensive use of synthetic organic chemical insecticides results in environmental hazards and resistance in major vector species and this has necessitated to develop a more potent and environmental friendly insecticide. Essential oils extracted from Brazilian plants exhibited larvicidal activity\(^12\) against Aedes aegypti with LC\(_{50}\) of 60-538 ppm. Aluminum chloride obtained from alder leaf is also reported to have larvicidal activity against A. aegypti\(^13\). Larvicidal activities of tannins and alkaloids have also been reported\(^14\)\(^,\)\(^15\).

In present study, two types of leaves exhibited good larvicidal activities on mosquito with varying susceptibility, which is in agreement with reports from previous findings\(^16\). Acetone extracts had pronounced larvicidal activity than aqueous extracts, indicating that active constituents of leaves have more ability to dissolve in acetone solvent than aqueous solvent as reported\(^7\) in their mosquito larvicidal constituents from Lautana viburnoides. S. monostachyus demonstrated a greater potential for larvicidal activity on A. gambiae than O. gratissimum, may be attributed to high content of saponin in S. monostachyus than O. gratissimum. Saponins are freely soluble in both organic solvents and water, and work by interacting with cuticle membrane of larvae ultimately disarranging the membrane, which is probable reason for larval death\(^17\). Many studies\(^3\)\(^,\)\(^8\) reported that saponins showed 100% mortality against mosquito larvae. LC\(_{50}\) of extracts of two types of leaves revealed pronounced larvicidal activities against A. gambiae. Since Anopheles mosquito breeds in drinking water tank, many of plant extracts are subject to risk factors in mosquito control. Plant extracts that are highly toxic against Anopheles mosquito are also toxic to human beings. In present study, S. monostachyus and O. gratissimum extracts showed high toxic effect on Anopheles mosquito larvae but they are non-toxic to human beings.

Conclusions

Larvicidal bioassays of O. gratissimum and S. monostachyus leaf extracts showed promising evidence in A. gambiae eradication. Larvicidal activity of S. monostachyus was found higher than that of O. gratissimum. Acetone extracts showed higher larvicidal activities than aqueous extracts. Thus leaf extracts have great potential for prevention of malaria spread and environmental pollution.

References

