

# LARVICIDAL ACTIVITY OF *SACOGLOTTIS GABONNENSIS* STEM BARK EXTRACTS AGAINST *CULEX QUINQUEFASCIATUS* AND *AEDES AEGYPTI* MOSQUITOES IN UYO, NIGERIA

## Abstract

The quest for alternatives to synthetic chemical insecticides against mosquito vectors has necessitated studies on natural plant products. The larvicidal effect of ethanolic and aqueous extract of a medicinal plant *Sacoglottis gabonnensis* stem bark were tested against 4<sup>th</sup> instar larvae of *Culex quinquefasciatus* and *Aedes aegypti*. Larval bioassays were carried out at different concentrations (0.45 – 0.75% w/v) of the extract in accordance with the WHO standard protocol. Mortality was recorded after 24 and 48 hours exposure. The LC<sub>50</sub> and LC<sub>90</sub> were also determined. The highest concentration (0.75% w/v) of the ethanolic extract of *S. gabonnensis* resulted in 80% mortality of *Culex quinquefasciatus* with LC<sub>50</sub> (2.59) and LC<sub>90</sub> (3.42) and 70% mortality of *Aedes aegypti* with LC<sub>50</sub> (1.22) and LC<sub>90</sub> (2.31). At a concentration of 0.75% w/v the aqueous extract of *S. gabonnensis* resulted in 65% mortality of *Culex quinquefasciatus* with LC<sub>50</sub> (2.84) and LC<sub>90</sub> (3.62) and 60% mortality of *Aedes aegypti* with LC<sub>50</sub>(2.16) and LC<sub>90</sub>(2.70). *Culex quinquefasciatus* was not significantly ( $X^2=1.66$ , df 1,  $P<0.05$ ) more susceptible to ethanolic extract of the plant than *Aedes aegypti*. The susceptibility of both mosquito species to the aqueous extract was also not significant ( $X^2=2.20$ , df 1,  $P>0.05$ ). This preliminary investigation has revealed that the stem bark of *S. gabonnensis* could be harnessed in the formulation of potent biocides against mosquito vector.

**Keywords:** Larvicidal, *Sacoglottis gabonnensis*, mosquitoes, Uyo, Nigeria

## INTRODUCTION

Vector-borne diseases remain a major cause of morbidity and mortality worldwide particularly in countries with tropical and subtropical climates. Mosquito-borne diseases such as filariasis, dengue fever, Japanese encephalitis, yellow fever, malaria zika virus disease among others continue to plague man [1].

*Culex* mosquitoes are vectors of filariasis, a disease which is a major health challenge in Nigeria and most of sub-saharan Africa. *Aedes* mosquitoes on the other hand are vectors of important viral diseases of man such as the zika, dengue and yellow fevers. *Aedes aegypti* is reported to be the principal vector of dengue fever in tropical countries [2]. Dengue fever is considered a serious public health problem world over, especially in areas where the environmental conditions for the development of *Aedes* mosquitoes abound.

Although mosquito-borne diseases represent a greater health problem in tropical and sub-tropical climates, no part of the world is completely free from the risk [3].

40 Different strategies have been adopted to reduce the prevalence and incidence of  
41 mosquito-borne diseases. One of the approaches for control of these diseases is the  
42 interruption of disease transmission either by killing adults, preventing contact with man or  
43 by causing larval mortality in the breeding sites [4]. Mosquito control is a difficult task and  
44 more so due to a variety of factors including the development of insecticide resistance and  
45 concern over environmental pollution [5]. As a result of resistance, conventional insecticides,  
46 the major agent for vector control are becoming ineffective [6]. Resistance vary in intensity  
47 with time, species in question as well as with location. Resistance against biopesticides such  
48 as *Bacillus sphaericus* have also been reported [1]. Areas with more agricultural activities are  
49 even more at risk as the use of agricultural insecticides increase the risk of resistance in  
50 disease vectors and non-target organisms. Among the approved classes of insecticide for  
51 public health use, the most widely exploited of them, DDT and the pyrethroids have reported  
52 highest level and widespread resistance from different mechanisms. There is presently a  
53 heightened demand for alternatives that are more environmentally friendly, less toxic and  
54 non-toxic to non-targets organisms, degradable and target specific.

55 Natural products of plant origin presents promising potentials for vector control. They  
56 are usually active against limited number of species, less expensive, readily biodegradable  
57 and non-toxic to non-target organisms and suitable for use in both agricultural and public  
58 health purposes. Extracts from plants sources have been shown to possess insecticidal  
59 properties [7]; [8]; [9].

60 One plant may possess bioactive and insecticidal properties with wide range of  
61 activities. *Sacoglottis gabonnensis* is a typical example. It has been shown to possess high  
62 medicinal value as it is used in treatment of many diseases as well as an insect growth  
63 regulator [10]. It is a low land, rainforest species found along the axis of Sierra Leone to  
64 Nigeria, and south-eastwards into Zaire and Angola.

65 There is paucity of data on the larvicidal properties of this plant especially on  
66 mosquitoes, in Nigeria, where mosquito-borne diseases is a major source of morbidity and  
67 mortality. The present study was therefore designed to evaluate the lavicidal potential of  
68 *Sacoglottis gabonnensis* on larvae of *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes.

## 69 MATERIALS AND METHODS

### 70 Collection of plant material

71 Stem bark of *Sacoglottis gabonnensis* was collected from Itak in Ikono Local  
72 Government Area, Akwa Ibom State, Nigeria. It was identified by a plant taxonomist in the

Department of Botany and Ecological Studies, University of Uyo, Nigeria. Thereafter a voucher specimen was deposited at the herbarium with identification number Ononiwu UUH013/12

## Extraction

The stem bark of *S. gabonnensis* was washed with distilled water, shade-dried and pulverized using a mechanical grinder (corona). The powder of the stem bark of the plant was obtained and 200g was weighed out for aqueous and ethanolic extractions using a soxhlet apparatus as described by [11]. The solvents from the extracts were removed using a rotary vacuum evaporator to collect the crude extract. The weight of each extract was determined and percentage yield (expressed as % w/w was noted).

## Larvicidal bioassay

The larvicidal properties of the plant crude extracts were assessed according to [12] protocol. Batches of 25 4<sup>th</sup> instar larvae of Aedes and Culex mosquitoes were separately transferred to small disposable test cups containing nutrient. The mosquito were exposed to different concentration (0.45%, 0.60%, 0.75% w/v) of each extract **respectively**. Five replicates were set up for each extract concentration. The controls were also replicated and each control set up had 25 larvae of each of the different mosquito species immersed separately in 100ml distilled water to which larvae food had been introduced. Both the test and control set ups were maintained at room temperature. Observations were made at 24 and 48 hours and larvicidal activity of each extract was determined by counting the number of dead larvae each day. Until the end of the experiment, larvae was considered dead when they did not move and did not respond to stimulus with a Pasteur pipette [13].

## Statistical Analysis

Data generated were analyzed using statistical package for social sciences (SPSS) version 20. The average larval mortality data were subjected to probit analysis for calculating LC<sub>50</sub> and LC<sub>90</sub> and other statistics at 95% confidence limits of upper confidence limit (UCL) and Lower Confidence limits (LCL) and chi-square.

## RESULTS

The percentage yields of the extracts used were 3.56% w/v for ethanolic and 5.03% for aqueous.

The activity of the larvae exposed to different test solutions reduced as shown in their slow wriggling and motility movement. This was more apparent as concentration of the

extract increased. The results of the larvicidal activity of ethanolic and aqueous extract of *S. gabonnensis* against the larvae of two important mosquitoes vectors viz: *Culex quinquefasciatus* and *Aedes aegypti* are presented in Tables 1 and 2.

The highest larvicidal activity was observed in ethanolic extract. The percentage mortality of *Culex quinquefasciatus* larvae treated with various concentrations of ethanolic and aqueous extract of *S. gabonnensis* after 48 hours is presented in table 1. The highest percentage (80%) mortality was recorded at a concentration of 0.75 w/v, while the least percentage (47.70%) mortality was recorded at 0.45 w/v. For the aqueous extract, the highest percentage (65.0%) mortality was noted at 0.75 w/v, while the least percentage (44%) was recorded at 0.45 w/v of the plant extract. The LC<sub>50</sub> and LC<sub>90</sub> for ethanolic and aqueous extract of *S. gabonnensis* after 48 hours were determined to be (2.59 and 1.99) and (2.84 and 1.85) respectively.

Table 2 shows the percentage mortality of *Aedes aegypti* larvae treated with various concentrations of *S. gabonnensis* plant extracts after 48 hours. Differences of the percentage mortality among the plants extracts were observed. The highest percentage (70%) mortality was recorded at a concentration of 0.75 w/v of the extract, while the least percentage (26%) mortality was recorded at a concentration of 0.45 w/v of the extract. The LC<sub>50</sub> and LC<sub>90</sub> for ethanolic and aqueous extract of *S. gabonnensis* after 48 hours were determined to be (1.22 and 2.31) and (2.16 and 2.70) respectively. The extracts of the plant had no significant larvicidal effect on either of the mosquito species. *Culex quinquefasciatus* was not significantly ( $X^2=1.66$ , df 1,  $P<0.05$ ) more susceptible to ethanolic extract of the plant than *Aedes aegypti*; the susceptibility of both mosquito species to the aqueous extract was also not significant ( $X^2=2.20$ , df 1,  $P>0.05$ ). However, there was reduction in wriggling activity and mortality of the larvae exposed to the different test solutions. This was more evident as the concentration of the extract increased. Larvae of both mosquito species in the control experiment registered no death throughout the duration of the experiment, some even metamorphosed into pupae.

## DISCUSSION

This present study have shown the larvicidal activity of extracts of *S. gabonnensis* against *Cu. quinquefasciatus* and *Ae. aegypti*. These findings are comparable to earlier reports of [14], [15] and [13]. Plant extract might have complex mixture of biocidal active compounds, including phenolics, terpenoids, flavonoids and alkaloids which may jointly or

independently contribute to mortality and delayed growth of larvae [16], these factors might have accounted for the high mortality of the larvae recorded in this study.

Interestingly *Cu. quinquefasciatus* were more susceptible to *S. gabonensis* than *Ae. aegypti*. The difference in susceptibility could be attributed to the difference in the physiological characteristics between the two groups of mosquito [17]. The high larvicidal activity against *Cu. quinquefasciatus* is quite encouraging considering the fact that they are vectors of filariasis amongst other diseases in sub-Saharan Africa. The bioactivity of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts, age of plant parts, solvents used in extraction and mosquito species [18]. These factors could have been responsible for the variations observed in mortality.

The plant extracts exhibited a concentration dependent activity against the mosquito larvae, since the percentage mortality were observed to increase with increasing concentration and time of exposure. This observation agrees with the report of [19]; Rene [9]; [20], [13]. The ethanolic extract of the plant were more potent than the aqueous extract on the larvae of the mosquito species in this present study. This is consistent with the work of [21], who stated that the activity of extracts on target species varies with respect to the plant parts from which they are extracted and solvent of extraction among other factors. It is not unlikely that the difference in potency observed in ethanolic and aqueous extract of *S. gabonensis* in this study might be due to aforementioned reasons. There was a drastic reduction in activity of larvae exposed to the different test solutions as evidenced by loss of wriggling and motility. These effects on behaviour may be due to presence of neurotoxic compounds in the plant extracts [22]. No behavioural changes were noticed in control group, they were agile and wriggling throughout the duration of the study.

Plant could be an alternative and veritable source of mosquito larvicides because they contain an array of bioactive chemicals that are generally free from harmful effects. The screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products, and stimulate local efforts to enhance public health [23].

Since this study has demonstrated the larvicidal properties of *S. gabonensis* against mosquito species, the phytochemicals of this plant could be exploited in the formulation of potent biocides or insecticides. Further analysis is required to isolate the active principles and its mode of action in inhibiting the developmental stages in *Cu. quinquefasciatus* and *Ae. aegypti*.

# REFERENCES

1. Govindarajan M, Rajeswary M, Amsath A. Larvicidal properties of *Caesalpinia pulcherrima* (Family: Fabaceae) against *Culex tritaeniorhynchus*, *Aedes albopictus*, and *Anopheles subpictus*. *International Journal of Pure and Applied Zoology*. 2013; 1(1): 15-23.
2. World Health Organization. Impact of dengue. WHO. 2001; Available at <http://www.who.int/cst/disease/dengue/impact/en/index.html>.
3. Fradin MS. and Day JF. Comparative efficacy of insect repellants against mosquito bite. *New England Journal of Medicine*. 2002; 347:13-18.
4. Rajmohan D. and Ramiaswamy M. Evaluation of larvicidal activity of the leaf extract of a weed plant, *Ageratina adenophora* against two important species of mosquito *Ae. Aegypti* and *Cx. quinquefasciatus*. *African Journal of Biotechnology*. 2007; 631-638.
5. Srivastava HC. and Sharma SK. Chloroquine resistant *Plasmodium falciparum* in migrant population. *Indian Journal of Malariology*. 2000; 37: 39-42.
6. Vincent I. Cycling to the finish: Natural pesticide from the neem tree and other tropical plants. Proceeding of the 2<sup>nd</sup> international neem conference, 1983, GTZ, Eschborn. 2000; 587.
7. Ojewole JAO, Rahim S. and Shode FO. Mosquito larvicidal properties of aqueous extracts of *Senna didymobotrya*. *Nigerian Journal of Natural Products Medicine*. 2000; 4:46-47.
8. Anyanwu GI. and Amefule EC. Comparative toxic effect of *Ocimum basilicum* (Labiatae), Citrus lemon (Rutaceae) and a conventional insecticide (coopex EC) on mosquito larvae. *West African Journal of Pharmacology and Drug Resistance*. 2001; 17:63-67.
9. Rene N, Oladimeji OH, Ubulom PME. and Ekpo BAJ. Larvicidal and antimicrobial potential of *Anthocleista djalensis* L. (Gentianaceae). *African Journal of Pharmaceutical Research and Development*. 2006; 2(1): 97-101.
10. Badre F. Humiriacea flore du Cameroun volume 14. Museum National d'Histoire Naturelle, Paris, France. 1972; 47-50.
11. Vogel AI. Textbook of practical organic chemistry. The English Language Book Society and Longman, London. 1978; 1368.
12. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Communicable disease control prevention and eradication. WHO Pesticide evaluation Scheme WHO, Geneva. 2005; WHO/CDS/WHOPES/GCDPP/1.3
13. Ubulom PME, Imandeh GN, Akpabio EE, Opara KN. and Ekanem MS. Larvicidal effect of aqueous and ethanolic extracts of *Senna alata* on *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes aegypti*. *Pakistan Journal of Pharmaceutical Science*. 2013; 26(3): 561-566.
14. Sakthivadivel M. and Daniel T. Evaluation of certain insecticidal plants for the control of vector mosquitoes viz, *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. *Applied Entomology and Zoology*. 2008; 43(1): 57-63.
15. Shivakumar MS, Srinivasan R, Narayan D. Larvicidal potential of some Indian medicinal plant extracts against *Aedes aegypti* (L). *Asian Journal of Pharmaceutical and Clinical Research*. 2013; 6(3): 77-80.
16. Varun J, Vadav R, Kumar SA, Vivek T, Yadav S, Vijay V, Sukumaran D. Larvicidal activity of leaf extract of some weeds against malaria vector *Anopheles stephensi*. *International Journal of Malaria Research and Reviews*. 2013; 1(3): 35-39.
17. Ohaga SO, Ndiege IO, Kabasu SS, Beier JC. and Mbogo CM. Larvicidal activity of *Piper guineense* and *Spilanthes mauritiana* Crude-powder against *Anopheles gambiae*

- 224 and *Culex quinquesfasciatus* in Kilifi district Kenya. *Journal of Biological Sciences*.  
 225 2007; 7(7): 1215-1220.
- 226 18. Syed-Ali M, Ravikumar S. & Margaret Beula J. Bioactivity of seagrass against the  
 227 dengue fever mosquito *Aedes aegypti* larvae. *Asian Pacific Journal of Tropical*  
 228 *Biomedicine*. 2012; 2(7): 570-573.
- 229 19. Obomanu FG, Ogbala OK, Gabriel UU, Fekarurhobo GK. and Adediran BI.  
 230 Larvicidal properties of *Lepidagathis alopecuroides* and *Azadirachta indica* on  
 231 *Anopheles gambiae* and *Culex quinquesfasciatus*. *African Journal of Biotechnology*.  
 232 2006; 5(9): 761-765.
- 233 20. Khanna VG. and Kannabiran K. Larvicidal effect of *Hemisdermus indicus*, *Gymnema*  
 234 *sylvestre* and *Eclipta prostrata* against *Culex quinquesfasciatus* mosquito larvae.  
 235 *African Journal of Biotechnology*. 2007; 6(3): 307-311.
- 236 21. Sukumar K, Perich MJ. and Boober IR. Botanical derivatives in mosquito control. A  
 237 *Review Journal of the American Mosquito Control Association*. 1991; 7: 210-212.
- 238 22. Rajasekaran A. and Duraikannan G. Larvicidal activity of plant extracts on *Aedes*  
 239 *aegypti*. *Asian Pacific Journal of Tropical Medicine(s)*. 2012; 1578-1582.
- 240 23. Bowers WS, Sener B, Evans PH, Bingol F. and Erdoogan I. Activity of Turkish  
 241 Medicinal Plants against mosquitoes *Aedes aegypti* and *Anopheles gambiae*.  
 242 *Insecticide Science Application*. 1995; 16(314): 339-342.

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244 **Table 1:** Larvicidal Activity of ethanolic and aqueous extract of *S. gabonnensis* on *Culex*  
 245 *quinquesfasciatus*

| 24 hours  |       |           |                  |                  | 48 hours |           |                  |                  |          |
|-----------|-------|-----------|------------------|------------------|----------|-----------|------------------|------------------|----------|
| Extract   | Conc. | %         | LC <sub>50</sub> | LC <sub>90</sub> | $\chi^2$ | %         | LC <sub>50</sub> | LC <sub>90</sub> | $\chi^2$ |
|           |       | Mortality | LCL-UCL          | LCL-UCL          |          | Mortality |                  |                  |          |
|           |       | ty        |                  | UCL              |          | ty        |                  |                  |          |
| Ethanolic | 0.00  | 0.00      |                  |                  | 0.007    | 0.00      | 2.59             | 1.99             | 0.00     |
|           | 0.45  | 30.00     | 6.59             | 5.94             |          | 47.70     | (-1.47-5.01)     | (-2.11-          |          |
|           | 0.60  | 29.00     | (-2.28-          | (-1.72-          |          | 59.00     |                  | 4.92)            |          |
|           | 0.75  | 40.00     | 4.49)            | 4.71)            |          | 80.00     |                  |                  |          |
|           | Conc. | %         | LC <sub>50</sub> | LC <sub>90</sub> | $\chi^2$ | %         | LC <sub>50</sub> | LC <sub>90</sub> | $\chi^2$ |
| Aqueous   |       | Mortality |                  |                  |          | Mortality |                  |                  |          |
|           |       | ty        |                  |                  |          | y         |                  |                  |          |
|           | 0.00  | 0.00      | 2.85             | 3.56             | 0.02     | 0.00      | 2.84             | 1.85             | 0.101    |
|           | 0.45  | 22.70     | (-1.60-          | (-1.86-          |          | 44.00     | (-1.64-4.93)     | (-2.01-          |          |
|           | 0.60  | 28.00     | 5.18)            | 2.88)            |          | 51.60     |                  | 3.91)            |          |
| 0.70      | 36.00 |           |                  |                  |          | 65.00     |                  |                  |          |

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251 **Table 2:** Larvicidal Activity of ethanolic and aqueous extract of *S. gabonnensis* on *Aedes*  
 252 *aegypti*

| 24 hours  |           |       |                  |                  | 48 hours  |       |                  |                  |          |
|-----------|-----------|-------|------------------|------------------|-----------|-------|------------------|------------------|----------|
| Extract   | Conc.     | %     | LC <sub>50</sub> | LC <sub>90</sub> | $\chi^2$  | %     | LC <sub>50</sub> | LC <sub>90</sub> | $\chi^2$ |
| Mortality |           |       |                  |                  | Mortality |       |                  |                  |          |
| Ethanolic | 0.00      | 0.00  |                  |                  |           | 0.00  |                  |                  |          |
|           | 0.45      | 10.00 | 1.59             | 1.99             |           | 26.00 | 1.22             | 2.31             |          |
|           | 0.60      | 20.00 | (0.60-7.01)      | (0.42-           | 0.00      | 45.30 | (0.67-6.01)      | (1.99-           | 0.212    |
|           | 0.75      | 29.30 |                  | 3.87)            |           | 70.00 |                  | 5.11)            |          |
|           | Conc.     | %     | LC <sub>50</sub> | LC <sub>90</sub> | $\chi^2$  | %     | LC <sub>50</sub> | LC <sub>90</sub> | $\chi^2$ |
| Aqueous   | Mortality |       |                  |                  | Mortality |       |                  |                  |          |
|           | 0.00      | 0.00  | 1.68             | 3.56             |           | 0.00  | 2.16             | 2.70             |          |
|           | 0.45      | 10.70 | (-0.411-         | (-0.97-          |           | 32.00 | (-1.03-5.67)     | (2.11-           | 0.29     |
|           | 0.60      | 17.30 | 7.22)            | 2.65)            |           | 42.00 |                  | 3.22)            |          |
|           | 0.75      | 30.70 |                  |                  |           | 60.00 |                  |                  |          |

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