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Larvicidal Activity of *Sacoglottis gabonnensis* Stem Bark Extracts against *Culex quinquefasciatus* and *Aedes aegypti* Mosquitoes in Uyo, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KNO, PMEU and NIU designed the study. Authors FMC and NEO were involved in the field study. Authors KNO, PMEU, NIU and FMC performed the statistical analysis, wrote the protocol and drafted the manuscript. Authors KNO, NEO and FMC managed the literature searches. All authors read and approved the final manuscript.

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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 extracts of a medicinal plant *Sacoglottis gabonnensis* stem bark were tested against 4th instar larvae of *Culex quinquefasciatus* and *Aedes aegypti*. Larval bioassays were carried out at different concentrations (0.45 – 0.75% w/v) of the extracts in accordance with the WHO standard protocol. Mortality was recorded after 24 and 48 exposure hours. The LC_{50} and LC_{90} were also determined. The highest concentration (0.75% w/v) of the ethanolic extract of *S. gabonnensis* resulted in 80% mortality of *Cu. quinquefasciatus* with LC_{50} (2.59) and LC_{90} (3.42) and 70% mortality of *Ae. aegypti* with LC_{50} (1.22) and LC_{90} (2.31). At a concentration of 0.75% w/v the aqueous extract of

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S. gabonennensis resulted in 65% mortality of *Cu. quinquefasciatus* with LC_{50} (2.84) and LC_{90} (3.62) and 60% mortality of *Ae. aegypti* with LC_{50} (2.16) and LC_{90} (2.70). *Cu. quinquefasciatus* was not significantly (χ^2=1.66, df 1, P>0.05) more susceptible to ethanolic extract of the plant than *Ae. aegypti.* The susceptibility of both mosquito species to the aqueous extract was also not significant (χ^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; mosquito; Uyo; Nigeria.

1. 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 virus, dengue and yellow fevers. *Ae. aegypti* is reported to be the principal vector of dengue fever in tropical countries [2], a disease 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].

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

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

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

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

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Stem bark of *S. gabonnensis* was collected from Itak in Ikono Local 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.

2.2 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/v was noted).

2.3 Larvicidal Bioassay

The larvicidal properties of the plant crude extracts were assessed according to [12] protocol. Batches of 25 fourth 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. 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 100 mL 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 were considered dead when they did not move and did not respond to stimulus with a Pasteur pipette [13].

2.4 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_{50} and LC_{90} and other statistics at 95% confidence limits of upper confidence limit (UCL) and Lower confidence limits (LCL) and chi-square.

3. 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. This was more apparent as concentration of the extract increased. The results of the larvicidal activity of ethanolic and aqueous extracts of *S. gabonnensis* against the larvae of two important mosquito vectors viz: *Cu. quinquefasciatus* and *Ae. aegypti* are presented in Tables 1 and 2.

The highest larvicidal activity was observed in the ethanolic extract. The percentage mortality of *Cu. 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_{50} and LC_{90} for ethanolic and aqueous extracts of *S. gabonnensis* after 48 hours were determined to be 2.59 and 1.99; and 2.84 and 1.85.

Table 2 shows the percentage mortality of *Ae. aegypti* larvae treated with various concentrations of *S. gabonnensis* plant extracts after 48 hours. Differences in percentage mortality with varying concentrations of the 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 ethanolic extract. The LC_{50} and LC_{90} for ethanolic and aqueous extracts of *S. gabonnensis* after 48 hours were determined to be 1.22 and 2.31; and 2.16 and 2.70. The extracts of the plant had significant larvicidal effect on both mosquito species. *Cu. quinquefasciatus* was not significantly (χ^2=1.66, df 1, P>0.05) more susceptible to ethanolic extract of the plant than *Ae. aegypti;* the susceptibility of both mosquito species to the aqueous extract was also not significant (χ^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. Few larvae metamorphosed into pupae in the control experiment.

Table 1. Larvicidal activity of ethanolic and aqueous extract of *S. gabonnensis* on *Cu. quinquefasciatus*

		24 hours				48 hours			
Extract	**Conc.**	**% mortality**	**LC_{50} LCL-UCL**	**LC_{90} LCL-UCL**	**χ^2**	**% mortality**	**LC_{50} LCL-UCL**	**LC_{90} LCL-UCL**	**χ^2**
Ethanolic	0.00	0.00				0.00	2.59	1.99	
	0.45	29.00	6.59	5.94		47.70	(-1.47-5.01)	(-2.11-4.92)	
	0.60	30.00	(-2.28-4.49)	(-1.72-4.71)	0.007	59.00			0.00
	0.75	40.00				80.00			
	Conc.	**% mortality**	**LC_{50}**	**LC_{90}**	**χ^2**	**% mortality**	**LC_{50}**	**LC_{90}**	**χ^2**
Aqueous	0.00	0.00	2.85	3.56		0.00	2.84	1.85	0.101
	0.45	22.70	(-1.60-5.18)	(-1.86-2.88)	0.028	44.00	(-1.64-4.93)	(-2.01-3.91)	
	0.60	28.00				51.60			
	0.75	36.00				65.00			

Table 2. Larvicidal activity of ethanolic and aqueous extract of *S. gabonnensis* on *Ae. aegypti*

		24 hours				48 hours			
Extract	**Conc.**	**% mortality**	**LC_{50} LCL-UCL**	**LC_{90} LCL-UCL**	**χ^2**	**% mortality**	**LC_{50} LCL-UCL**	**LC_{90} LCL-UCL**	**χ^2**
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)	(0.42-3.87)	0.00	45.30	(0.67-6.01)	(1.99-5.11)	0.212
	0.75	29.30				70.00			
	Conc.	**% mortality**	**LC_{50}**	**LC_{90}**	**χ^2**	**% mortality**	**LC_{50}**	**LC_{90}**	**χ^2**
Aqueous	0.00	0.00	1.68	3.56		0.00	2.16	2.70	
	0.45	10.70	(-0.411-7.22)	(-0.97-2.65)		32.00	(-1.03-5.67)	(2.11-3.22)	0.29
	0.60	17.30				42.00			
	0.75	30.70				60.00			

4. DISCUSSION

This present study has shown the larvicidal activity of extracts of *S. gabonnensis* against *Cu. quinquefasciatus* and *Ae. aegypti*. These findings are comparable to earlier reports of [13,14]; and [15]. Plant extracts might have complex mixture of bioactive 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* larvae were more susceptible to *S. gabonennsis* than *Ae. aegypti*. The difference in susceptibility could be attributed to the difference in the physiological characteristics between the two species 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 mosquito larvae, since percentage mortality was observed to increase with increasing concentration and time of exposure. This observation agrees with the reports of [9,13,19,20]. The ethanolic extract of the plant was 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. gabonnensis* 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 the presence of neurotoxic compounds in the plant extracts [22]. No behavioural changes were noticed in the control group, they were agile and wriggling throughout the duration of the study.

Plants could be an alternative and veritable source of mosquito larvicides because they contain an array of bioactive chemicals. 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].

5. CONCLUSION

This study has demonstrated the larvicidal activities of *S. gabonnensis* 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 ingredients and decipher the mode of action in inhibiting the development of immature stages of *Cu. quinquefasciatus* and *Ae. aegypti*.

CONSENT AND ETHICAL APPROVAL

Not applicable because the study was not based on any human, mammalian or avian subject.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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