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Larvicidal Efficacies, Insect Growth Regulatory Activities and Vectorial Fitness of *Sterculia setigera* Bark Extracts against *Culex quinquefasciatus* Mosquitoes - Diptera: Culicidae

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Abstract

Mosquitoes are not only a nuisance as biting insects; they are also involved periodically in transmitting diseases to human and animals. Frequent use of insecticides for mosquito control has caused development of resistance, adverse effect on non –target organisms and serious environmental concern. This study was conducted to determine the phytochemical constituents of bark (S.*setigera)* and evaluate its vectorial fitness potential, insect growth regulatory efficacies and mosquito larvicidal activities against *Culex quiquefasiatus*. The egg rafts of *culex quinquefasciatus* mosquitoes were collected from some breeder habitat within the study area and were incubated using standard protocol. The barks of the plant were extracted using methanol and n-hexane. Four concentrations 0.00625, 0.0125, 0.025 and 0.05 were used for n-hexane and 0.2, 0.3, 0.4 and 0.5 were used for methanol respectively. The test procedure was carried out according to World Health Organisation protocol for insecticide testing. The bark of *S.setigera* reveals important bio-active phytochemicals namely; flavonoids, tannins, saponins, alkaloid, steroid, anthraquinone, and cardiac glycoside. The methanol and n-hexane of the bark of *S.setigera* significantly elongated the developmental survivorship of *Culex quinquefasciatus.* The results obtained for larvicidal bioassay of methanol and n-hexane extracts of the bark of *Sterculia setigera* showed significant ($p<0.05$) effect on the species. The results also indicated a dose-and – time dependent toxicity of the plant. i.e. the higher the concentration and period of time, the higher the mortality. The LC_{50} and LC_{90} of the methanol extracts of the bark of *Sterculia setigera* were 0.25mg/L and 0.42mg/L While LC_{50} and LC_{90} of the bark of n-hexane extract were 0.01mg/L and 0.04mg/L respectively. The results of this study, suggested that the plant species, *S.setigera*, possess promising bio-active phytochemicals for larvicidal and growth disruptive agents for effective mosquito vector control.

Keywords: Vectorial Fitness, Larvicidal, *S.setigera*, Phytochemical and Insect growth regulatory.

Introduction

Despite the giant stride recorded against mosquitoes in the last few decades, the vector remained a major public health threat, worldwide and has been appreciated as the greatest notoriety among arthropods. (1). According to (2) more than 100 species of mosquitoes have potentials to vector various diseases in human and other vertebrates. Their danger contributes significantly to diseases burden, death, poverty and social debility all over the world, especially in Africa (Nigeria), south of the Sahara. For instance, *C. quinquefasciatus* is a multiracial mosquito with worldwide distribution and sub-tropical areas and is associated with human dwellings. (3). *C. quinquefasciatus*, the southern house mosquito, has been relatively well studied in recent years probably because of their role in the transmission of important human diseases such as urban lymphatic filariasis, saint Louis encephalitis virus and western equine encephalitis virus (4).

Sterculia setigera commonly known as karaya gum plant locally called igi ose' one of the plants used in traditional medicine. It is a member of the family *Sterculiaceae*, a multipurpose savannah tree with a wide ecological spread in tropical Africa. It is found mostly in the wild (5). It is a deciduous plant that grows up to 40 feet high, with a smooth bark, and it peels off thin scales to expose yellowish patches (6). This plant has been used in traditional medical practises by various communities in Africa (7). *Sterculia* species are rich in alkaloids, saponins, and flavonoid glycosides, which showed a wide array of biological activities such as antimicrobial, antifungal, insecticidal, cytotoxic, antioxidant and anti-inflammatory activities (8). The gum is used in the treatment of snake bites, leprosy, syphilis, coughs, bronchitis and rickets and to manage insanity. (6) Also reported that this plant

contains active metabolites such as tannins, flavonoids Saponnins and glycosides. These gummy exudates are also used as stabilizers, disintegrators, and active ingredient release enhancers. (9) Hence, Theaim of this research, hence, is to study the effect of bark of *S.setigera* for larvicidal efficacies, vectorial fitness, development and duration of *culex quinquefasciatus*.

Materials and Methods

Description of study area

The mosquitoes were collected from their breeding habitat in bosso dam and the study was carried out in Minna, the capital of Niger State, Nigeria. Minna is located within Longitude 6° 33'E and Latitude 9° 37'N, covering a land area of 88km2 with an estimated human population of 1.2 million (The Nigeria Congress, 2007). The area has a tropical climate with mean annual temperature, relative humidity and rainfall of 30.2°C, 61% and 1334mm, respectively. The climate presents two distinct seasons; a rainy season between April and October, with highest mean monthly rainfall in September, and a dry season (November-March) completely devoid of rains. Its vegetation is typically grassing dominated savannah with scattered short trees. Minna has three main ethic groups namely Gwari's, Nupe's and Hausa's; and their major occupation is farming (10).

Collection and processing of plant material

The barks of the plant of *sterculia setigera* were collected from the field in Bosso area of Niger State and authenticated by a Senior Botanist in the Department of plant Biology, FUT, Minna. The barks were shade dried at room temperature. Thereafter, pulverized using a blender (11).

Preparation of plant extracts

The dried powdered barks of the plant were extracted using Soxhlet's apparatus and solvents used were N-hexane and methanol. Each of the milled plant, 50g was weighted with electric weighing balance and wrapped in a filter paper using a stapler to hold it. The weighed powdered plant material was then placed in the extracting flask of Soxhlet's apparatus, after which 300ml of N-hexane and 250mL of methanol was added to the plant material in a round bottom flask of the Soxhlet set up and set at 60^{O}C, after which the crude extracts were preserved in the refrigerator at 40^{0}C until needed for bio-assay (11).

Collection of mosquito egg rafts

Egg rafts were collected from stagnant water bodies in Bosso Market using a scoop and a bowl. After collection the egg rafts were brought to the Laboratory of the Department of Biological Sciences, FUT Minna. Each of the egg rafts was transferred to a one litre capacity bowl with 500ml of distilled water for hatching. The larvae were fed with fish feed. The feeding and changing of their rearing continued until the larvae develops into the 4th instars (L4) larva stage. This is in line with the techniques of (12).

Preparation of stock and working solutions

Analytical grade of methanol and n-hexane was used and 1g of each of the extracts was weighed with an electric weighing balance and 10ml of the solvent (methanol or N-hexane) was added to it. To prepare the stock solution, each extract was dissolved in the solvent that was used in extracting it, and the working solution was prepared by adding 90mL of distilled water to 10mL of the stock solution. A positive control containing (distilled water + solvents) and Negative control containing 100 % distilled water; each in 4 replicates. Subsequently, test concentrations ranging from 0.00625 % to 0.05 % n-hexane and for methanol 0.1 to 0.4 % were obtained from the working solution and also, four replicate were used for each test concentrations (12).

Phytochemical screening of S. setigera

Qualitative phytochemical screenings of the crude extracts were carried out using the standard procedure described by (13) and (14).

Data management and analysis

The data obtained from this study were analysed using Statically Package for Social Science version 20.0. The data were initially processed using Microsoft excel 2007 version and expressed as mean ±standard error of mean, for each dose level. Statistical analysis was undertaken by One-way Analysis of Variance (ANOVA), coupled with Duncan Multiple Range Test (DMRT) to compare results between doses and among treatment and Control groups. For the calculation of the LC50 and LC90 values, the data were subjected to probit regression analysis. The result with $P<0.05$ were considered to be statistically significant.

Results and Discussion

Phytochemical screening of crude extracts of the barks of S.setigera

The bioactive metabolites of the crude extracts of the bark include flavonoids tannins, saponins, phlobtanin, steroids, alkaloids, cardiac glycoside, terpenoids, and anthraquinones. (Table 1)

Table 1: Phytochemical Composition of N- hexane and Methanol Bark Extracts of *S.setigera*

Phytochemical component	n-hexane extracts	Methanol extracts
Flavonoids	+	+
Tannins	+	+
Saponin	+	+
Alkaloids	+	+
Phlobtanin	+	–
Steroids	+	–
Cardiac glycosides	+	+
Anthraquinones	+	+
Terpernoids	–	–

All metabolite components were present in the n-hexane extracts, except terpenoids. Cardiac glycoside, flavonoids, tannins, alkaloids and anthraquinone were all present, while phlobtanin, steroids and terpernoids were absent in the methanolic extract.

Lavicidal activities of bark extract of Sterculia setigera

The extracts were found to possess significant lethality against the fourth instar larval stage of the mosquito species. The lethality of the two extracts revealed that the mortality of the larvae was concentration and time-dependent, i.e., higher mortality was achieved at high extract concentrations and with increase in duration of exposure. For the methanolic extract, 0.4 mg/L elicited 100 % (30.00_+0.00) at 24 hours, while similar mortality result was obtained for the 0.5 mg/L concentration within 12 hours (Table 2).

Table 2: Larvicidal activities of methanolic Bark extract of *Sterculia setigera* against 4th instar larvae of *Culex quinquefasciatus* Mosquito after 24 hours of exposure

Extract concentration (%)	-ve Control	+ve Control	0.4mg/L	0.5 mg/L
0mins	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
10mins	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.25±0.25^{a}
15mins	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	2.25±0.48^{b}
20mins	0.00±0.00^{a}	0.00±0.00^{a}	1.25±0.75^{b}	4.00±1.47^{c}
30mins	0.00±0.00^{a}	0.25±0.25^{a}	3.00±1.08c	9.00±1.29^{c}
1hr	0.00±0.00^{a}	0.25±0.25^{a}	7.25±0.48^{d}	12.00±1.41^{d}
2hrs	0.00±0.00^{a}	0.25±0.25^{a}	12.00±2.12^{c}	20.75±1.10^{e}
3hrs	0.50±0.50^{a}	0.25±0.25^{a}	16.00±1.68^{c}	22.50±0.96^{d}
6hrs	1.25±0.48^{b}	0.25±0.25^{a}	19.75±1.03^{d}	27.75±1.11^{e}
12hrs	1.25±0.48^{b}	0.25±0.25^{a}	25.00±0.41^{d}	30.00±0.00^{e}
18hrs	1.25±0.48^{b}	0.25±0.25^{a}	29.75±0.25^{c}	30.00±0.25^{c}
24hrs	1.25±0.48^{b}	0.25±0.25^{a}	30.00±0.00^{e}	30.00±0.00^{e}

Table 3: Larvicidal activities of N-hexane Bark extract of *Sterculia setigera* against 4th instar larvae of *Culex*

Extract concentration (%)	-ve Control	+ve Control	0.025 m/L	0.05 m/L
0mins	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
5mins	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.25±0.25[a]
10mins	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	1.00±0.58[b]
15mins	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	2.25±1.31[b]
20mins	0.00±0.00[a]	0.00±0.00[a]	1.50±0.05[b]	3.25±1.44[c]
30mins	0.00±0.00[a]	0.00±0.00[a]	3.25±0.63[c]	5.25±1.31[d]
1hr	0.00±0.00[a]	0.25±0.25[a]	5.75±1.11[d]	9.75±0.63[e]
2hrs	0.00±0.00[a]	0.25±0.25[a]	8.50±1.19[d]	12.50±1.71[e]
3hrs	0.00±0.00[a]	0.25±0.25[a]	11.75±1.44[d]	18.00±2.48[e]
6hrs	0.00±0.00[a]	0.75±0.48[b]	18.00±4.02[d]	24.00±3.24[e]
12hrs	0.00±0.00[a]	0.75±0.48[b]	26.75±2.66[e]	30.00±170[f]
18hrs	0.00±0.00[a]	0.75±0.48[b]	30.00±2.90[e]	30.00±1.50[f]
24hrs	0.00±0.00[a]	0.75±0.48[b]	30.00±1.31[e]	30.00±0.25[f]

***quinquefasciatus* Mosquito after 24 hours of exposure**

Values followed by different superscripts in the same column, are significantly different at P<0.05 level of significance. Similarly, in the n-hexane extract, 0.025 mg/L caused 100% (30 mosquitoes) mortality at the 18th hour, while similar absolute toxicity was achieved for 0.05 mg/L within 6 hours of the 24 hour of exposure (Table 3).

Table 4: Median (LC_{50}) and upper (LC_{90}) lethal concentrations of the n-hexane and methanol bark extracts of *Sterculia setigera*

		LC_{50}	LC_{90}	R^2	Regression equation
Methanol	Bark	0.25	0.42	0.8808	Y=231.71x-8.191
n-Hexane	Bark	0.01	0.04	0.628	Y=1502.6x+37.283

The lethal concentrations of the methanol and n-hexane bark were, respectively, 0.25 and 0.42 mg/L and 0.01 and 0.04 mg/L for the LC_{50} and LC_{90} (Table 4).

Insect Regulatory growth activities of bark extracts of *S.setigera* against *Cx. quiquefasciatus*

There were no significant differences or effect on both crude extract on the survivorship of the mosquito species. Average Larval and Survivorship were respectively 99.58±0.16 and 95.51 ±1.84 % and 99.49±0.25 and 96.14 ±1.34 % for n-hexane and methanol extract, respectively as shown in Table 5.

Table 5: Survival rate (%) of immature life stages of *Culex quinquefasciatus* mosquitoes exposed to sub-lethal concentration of methanol and n-hexane bark extract *Sterculia setigera*

	Extract concentration (%)	L1	L2	L3	L4	ALS	Pupae	ALS
Negative control	100% distilled water	99.58±0.42^{b}	97.90±1.08^{c}	100.00±0.00^{b}	100.00±0.00^{c}	99.37±0.31^{b}	100.00±0.00^{b}	96.99±2.63^{b}
Positve control (methanol)	0.025	100.00±.00^{b}	99.16±0.83^{c}	100.00±0.00^{b}	99.16±0.51^{c}	99.58±0.22^{b}	100.43±0.43^{b}	99.75±0.18^{b}
positive control(n-hexane)	0.003125	100.00±0.00^{b}	99.53±0.41^{c}	100.00±0.00^{b}	100.00±0.00^{c}	99.89±0.10^{b}	99.58±0.41^{b}	99.83±0.10^{b}
Methanol	0.025	100.00±0.00^{b}	88.29±17.10^{b}	100.56±1.14^{b}	97.34±1.83^{c}	97.19±2.82^{ab}	100.00±0.00^{b}	96.91±1.91^{b}
n-Hexane	0.003125	70.41±12.31^{a}	55.97±8.10^{a}	75.86±10.93^{a}	93.75±6.25^{a}	74.00±2.94^{a}	98.43±1.56^{a}	81.38±3.57^{a}

Values followed by the different superscripts in the same column, are significantly different at P<0.05 level of significance.

Table 6: Effect of sub-lethal concentrations of *Sterculia setigera* bark extracts on duration of development (days) of *Culex quinquefasciatus* mosquitoes

	Extract concentration (%)	L1	L2	L3	L4	TLD	Pupae	TOTAL DAY
Negative control	100% distilled water	1.41±0.03^{c}	1.57±0.01^{b}	1.88±0.05^{a}	2.37±0.11^{a}	7.24±0.13^{a}	2.24±0.08^{a}	9.49±0.21^{a}
Positive control(Methanol)	0.025	1.09±0.06^{a}	1.68±0.02^{c}	2.22±0.09^{b}	2.72±0.12^{b}	7.73±0.13^{a}	2.27±0.05^{a}	10.00±0.18^{a}
positive control (n-hexane	0.003125	1.20±0.11^{b}	1.50±0.04^{a}	2.22±0.03^{b}	2.70±0.11^{b}	7.74±0.29^{a}	2.46±0.71^{b}	10.20±0.15^{a}
Methanol	0.025	1.27±0.03^{b}	1.69±0.05^{c}	2.25±0.05^{b}	3.03±0.09^{c}	8.26±0.11^{b}	2.57±0.09^{b}	10.88±0.18^{ab}
n-Hexane	0.003125	1.23±0.09^{b}	2.12±0.08^{d}	2.57±0.09^{b}	3.04±0.11^{c}	8.96±0.26^{c}	2.52±0.10^{b}	11.49±0.32^{b}

Values followed by the different superscripts in the same column, are significantly different at P<0.05 level of significance.

From Table 6, the duration of development on the other hand, was significantly affected by the plant extracts and the total larval duration development was delayed from 7.24±0.13 days, in the negative control, 8.96±0.26, 8.26±0.11 days, respectively, in n-hexane and methanol bark extracts respectively. Similarly, pupal stage duration of development increased from 2.24±0.08 days, in the negative control, to 2.57±0.09 and 2.52±0.10 days, respectively, in methanol and n-hexane bark extracts.

There was significant increase in total immature duration development from 9.49±0.21 days to 11.49±0.32 and 10.88±0.18 days, respectively, both in n-hexane and methanol bark extracts. In Table 7, the effect of sub-lethal concentrations of *Sterculia setigera* bark extracts on the wing length and width measurement of *Culex quinquefasciatus* mosquitoes. It was revealed from the result that the wing length of the female right and left wing were the same (3.01 mm) and higher than the male left (2.91mm) and male right (2.94mm) respectively for n-

hexane bark extracts. The width of the female left and right were also the same (0.59 mm), but the male left width (0.59 mm) was higher than the male right width (0.58mm). The surface area of the female left and right (1.78mm^2) were the same and higher than the male left (1.72 mm^2) and male right (1.71 mm^2). Similarly, the volume of the female left and right (27.27 mm^3) were the same and higher than the male left (24.64 mm) and the male right (25.41 mm^3). The female wing lengths were found to be more symmetrical than the male.

For the methanol bark extracts, female right-wing length (3.04 mm) was higher than the female left (3.01 mm) while the male left-wing length (2.84 mm) was higher than the male right-wing length (2.82 mm). It was observed that both the female and the male wing length were not symmetrical. The width of the male left and right (0.60 mm) were the same and higher than the female left and right width (0.58 mm). The female left volume (27.27 mm) and the female right volume (28.09 mm) were higher than both the male left volume (22.91 mm3) and the male right volume (22.43 mm). Methanol bark extracts therefore have effect on the fluctuating asymmetry of *Culex quinquefasciatus* mosquitoes.

Table 7: Effect of sub-lethal concentrations of *Sterculia setigera* bark extracts on the wing length and width measurement of *Culex quinquefasciatus* mosquitoes

	0.03125(N-HEXANE)				0.1 (methanol			
	Female left	Female right	Male left	Male right	Female left	Female right	Male left	Male right
Length	3.01±0.01	3.01±0.01	2.91±0.02	2.94±0.01	3.01±0.02	3.04±0.03	2.84±0.03	2.82±0.02
Width	0.59±0.02	0.59±0.02	0.59±0.01	0.58±0.02	0.58±0.01	0.58±0.01	0.60±0.02	0.60±0.01
Wing surface area	1.78±0.04	1.78±0.04	1.72±0.02	1.71±0.02	1.75±0.02	1.76±0.03	1.70±0.05	1.69±0.03
Fluctuating asymmetry	0.00±0.00	0.00±0.00	0.01±0.03	0.01±0.03	0.02±0.01	0.01±0.04	0.01±0.04	0.01±0.05
Wing length cased	27.27±0.00	27.27±0.00	24.64±0.00	25.41±0.00	27.27±0.00	28.09±0.00	22.91±0.00	22.43±0.00

The results procured from this study revealed that the phytochemical constituents of *Sterculia setigera* contained significant levels of tannins, saponnins, anthraquinones and cardiac glycosides. This is in agreement with previous studies of (6). The presence of saponnins (although in low concentration) is an important indicator that justifies the use of *S.setigera* in traditional medicine. This is due to the fact that saponnins serves mainly as an important adjuvant in vaccines (15). According to (16). Bacterial properties of plant are attributed to the presence of active secondary metabolites such as alkaloids, flavonoids, and saponnins also important medicinal phytochemicals that could be used in place of synthetic insecticides in mosquito control programmes. This result is similar to the findings of (17), which reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant_ derived secondary materials, such as, terpenes, alkaloids, steroids and flavonoids.

Similarly, the results obtained from larvicidal bioassay activities of both the bark of *Sterculia setigera* extracts of methanol and n-hexane solvents, tested against *Culex quinquefasciatus* showed that both the organic extract of bark of *Sterculia setigera* are responsible and effective in killing the larvae of *Culex quinquefasciatus*. This is obvious from the fact that more than 90% or 100% of the larvae, in some situations, died at high concentrations of the extracts. The result from this study reveals that increase in the concentration of the extract results in increase in larvae mortality as compared to both the negative and positive control experiments. The mortality rate of the larvae was dependent on the concentration of the plant extracts and their period of exposure. This is because high extract concentration increases the toxicity potential of the plant which leads to the high mortality of the larvae as suggested by (18). Also, plant bioactive chemical is generally considered as nontoxic, easily biodegradable and show broad-spectrum target-specific activities against different species of vector mosquitoes (19).

In the present study, although, survivorship of immature life stages of *Cxquiquesfasciatus* was not affected by sub-lethal concentration of the plant extracts, i.e; the extracts show good insect growth regulatory (IGR) efficacy against *culex quiquefasciatus*, by eliciting significant alterations in immature developmental and adult vectoral fitness attributes. The sub lethal concentration of the extract increased duration of development significantly and reduced survival rate by >70%. This effect may be because the disruption of the endocrine mechanism that regulate ecdysis and metamorphosis, as previously suggested for neem seed kernel extracts (20). The significant reduction in longevity of Cx.*quiquefasciatus* mosquitoes that emerged as adults reveals that the sub-lethal effects of *S.setigera* bark-extract was successfully carried over from the immature to adult stage. This finding is very pivotal with respect to the vectoral capacity in the average life-span of a mosquito population equally reduces its life-time disease transmission potential, perhaps, to a level that can no longer sustain transmission (21). Surprising, the sub-lethal concentration of the extract had no significant effect on the wing symmetry, probably, indicating that the body organs of the test mosquitoes were optimally formed, despite exposure to the extract. Wing symmetry is a reflection of quality of insect body (22), including those directly related to vector competence and potential such as permeability of the walls of the salivary glands, viability of the ovaries, etc.

Conclusion

This study evaluates the mosquito larvicidal efficacies and insect growth regulatory potentials of *S.setigera* against *Culex quiquefasciatus*. The result of the phytochemical constituents of *Sterculia setigera* shows that the plant contains important medicinal phytochemicals that may be widely used in place of synthetic insecticides in mosquito control programmes. The results of larvicidal bioassay activities of the bark of *Sterculia setigera* extracts of methanol and n-hexane solvents tested against *Culex quinquefasciatus* and the results shows that the organic extract of the bark of *Sterculia setigera* are bioactive and effective in killing the larvae of *Culex quinquefasciatus*. However, further studies are necessary to evaluate the potency of extracts from other flora part of *S.setigera* such as the seed etc. as well as employ multiple extraction method. Also, further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species. The active ingredients of the extract responsible for larvicidal and adult emergence inhibition activity in *Culex quinquefasciatus* should also be identified and utilized, if possible, in preparing a commercial product/formulation to be used as a mosquitocide.

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