Larvicidal effect of aqueous and ethanolic extracts of *Senna alata on Anopheles gambiae, Culex quinquefasciatus* and *Aedes aegypti*

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Abstract: *Senna alata* is locally used in South Eastern Nigeria in the treatment of several infections which include ringworm and other parasitic skin diseases.The larvicidal activities of aqueous and ethanolic leaf and stem extracts of *S. alata* were evaluated in static bioassays, on fourth instar larvae of *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes aegypti*, at extract concentrations of 0.15, 0.30, 0.45, 0.60 and 0.75% w/v, for 72 hours. Mortality of larvae exposed to the different extracts increased with increase in extract concentration and time of exposure. This study revealed a differential potency of the extracts used and a difference in susceptibility of larvae to the extracts as evident by the 72hLC_{50} values obtained. The leaf extract proved to be more lethal to the larvae than the stem extract as judged by the 72hLC_{50} values obtained both for the aqueous as well as the ethanolic extracts assayed. Phytochemical screening of the plant parts investigated revealed the presence of some plant metabolites, which have been reported in separate studies to be lethal to mosquito larvae. Results obtained from this study suggest that the leaf and stem extracts of *S. alata* possess a promising larvicidal potential which can be exploited in mosquito vector control.

Keywords: Larvicidal, *Senna alata*, extracts, *Anopheles gambiae, Culex quinquefasciatus, Ae. aegypti*.

INTRODUCTION

Our world today is still plagued by a myriad of ailments/diseases and a number of these diseases are caused by organisms which are vector-borne. Mosquitoes which also serve as vectors of diseases to humans, have a world wide distribution. They occur throughout the tropical and temperate regions and constitute a major public health menace. The mosquito, *Anopheles gambiae* has been incriminated with several disease-causing organisms, notably *Plasmodium spp* responsible for the notorious malaria scourge. Malaria is one of the major killer diseases of the world especially in Sub-Saharan Africa. Pharmaceutical brands have been assaulting malaria with qualified success over the past decades, yet the deadly fever fights back with the ferocity of a wounded lion (Alabi, 2010). *An. gambiae* is also a vector of the filarial nematode, responsible for filariasis. *Culex quinquefasciatus* is one of the vectors of the filarial worm, *Wuchereria bancrofti*, which is responsible for bancroftian filariasis, a disease which is a major health challenge. The diseases, yellow fever and dengue to mention a few are caused by organisms which are vectored by *Aedes aegypti*. This insect species is reported to be the principal insect responsible for dengue fever transmission in the tropical countries (WHO, 2001).

Different strategies have been devised to reduce the prevalence of insect-borne diseases, but these strategies have their limitations. One such strategy is the use of synthetic insecticides which has suffered from major disadvantages of environmental pollution and physiological resistance by vectors. These limitations have created the need for environmentally safe, degradable and target-specific insecticides against these insect vectors. The search for such compounds has been directed extensively to the plant kingdom. Extracts from plant sources have been shown to posses insecticidal properties (Ojewole *et al.*, 2000; Anyanwu and Amefule, 2001; Rene *et al.*, 2006 and Khanna and Kannabiran, 2007). Obomanu *et al.* (2006), have reported that these natural insecticides are often less toxic and don't accumulate chemical residues in flora, fauna and soil. Besides they can be obtained by individuals and communities easily at a very low cost. It is on this premise that the larvicidal potential of *Senna alata* (Leguminosae: Caesalpiniodeae) was investigated.

S. alata is an ornamental shrub, with a height of about 1-2m. It produces pretty yellow flowers in a column that resembles yellow candlesticks. In Nigeria, it is used for the treatment of several infections (Etukudo, 2003). Related species to *S. alata* (*Senna occidentale* and *Senna alecandrina*) were reported by Fafioye (2005) to have piscicidal potential. Ojewole *et al.* (2000), reported on the mosquito larvicidal property of *Senna didymobotrya*, but there is a paucity of scientific information on the larvicidal/insecticidal properties of *S. alata*.

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This study was designed with the aim of investigating the larvicidal potential of aqueous and ethanolic extracts of the leaf and stem of *S. alata* on fourth instar larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti.*

MATERIALS AND METHODS

Preparation of plant material

S.alata was collected from Midim area of Abak Local Government Area, of Akwa Ibom State, Nigeria. It was identified by Dr. Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo, Nigeria, with herbarium no. Ubulom UUH 874. The different parts of the plant were washed separately with distilled water and then air-dried on laboratory tables for about two weeks. After air-drying each plant part was separately pulverized using the crusher machines (Atlas exclusive, type YL and Gondard 77260, type TN 20), in the pilot plant unit of the Department of Medicinal Plant Research and Traditional Medicine (MPRTM), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. Plant extracts were obtained by maceration in 50% ethanol, for ethanolic extracts and distilled water for aqueous extracts. Maceration lasted for 72h with periodic stirring. This was followed by repeated filtration using muslin cloth, non-absorbent cotton wool and Whatman No.1 filter paper, and after filtration each liquid extract was concentrated. Aqueous extracts were concentrated using a lyophilizer (Amsco/Finn-Aqua lyovac, Germany). For ethanolic extracts, ethanol was first evaporated using a rotary evaporator (Buchi Laboratoriums Technik AG, CH 9230) at a temperature of 40^0C, before freeze drying. The weight of each extract was determined and the percentage yield (expressed as % w/w) was noted.

Phytochemical screening

The plant parts were investigated for the following plant metabolites: alkaloids, anthraquinones (free and combined), cardiac glycosides, flavonoids, saponins, tannins, phlobatannins and terpenes. Standard phytochemical methods described by Harborne (1984), Evans (2002) and Sofowora (2006) were employed.

Bioassay for larvicidal activity

Between April 2007 and September 2009 the larvicidal potentials of the leaf and stem of *S. alata* were evaluated. Fourth instar larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti* used in this study were provided by the National Arbovirus and Vectors Research Centre (NAVRC), Enugu, Nigeria. Also some larvae of *An. gambiae* were obtained from Nigeria Institute for Medical Research (NIMR), Lagos, as eggs on cloth paddles. These were hatched and maintained in the insectary until they reached their fourth instair, before assays were carried out. All assays were carried out in the Entomology unit of NAVRC, Enugu. The method employed for the determination of larvicidal activity was adopted from that described by Ojewole *et al.* (2000) and WHO (2005). A stock solution of 5% w/v of the extract was prepared and heated in a water bath at a temperature of 40^oC for two minutes, after which it was left to cool for about thirty minutes. From this stock solution graded concentrations of extracts of the leaf and stem were prepared to obtain 0.15, 0.30, 0.45, 0.60 and 0.75% w/v concentrations for each extract. Twenty five larvae were exposed to each bioassay medium in a final volume of 100ml formulation, taken in plastic assay cups containing nutrients (a pinch each of fine quaker oats). In other words, twenty five larvae of *An. gambiae* were exposed to each test solution. The same was done using larvae of *Cu. quinquefasciatus* and *Ae. aegypti*. 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 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 (28 ± 2^oC).

Observations were made at 24, 48 and 72h 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.

RESULTS

The percentage yields of extracts used were 8.30 and 5.65%w/w for aqueous leaf and stem extracts respectively and 6.17 and 3.58% w/w for ethanolic leaf and stem respectively.

Alkaloids, anthraquinones, cardiac glycosides flavonoids, saponins, tannins, phlobatannins and terpenes were detected in the leaves of *S.alata,* while anthraquinones, cardiac glycosides, flavonoids, tannins and terpenes were detected in the stem. Saponins were detected in the stem when the froth test was carried out.

Generally, there was reduction in activity (e.g. wriggling/motility) of larvae exposed to the different test solutions. This was more apparent as concentration of extract increased. Larvae of all mosquito species in the control experiments registered no death throughout the duration of the experiment and a few even metamorphosed into pupae. Also mortality of larvae exposed to the extracts increased with increase in extract concentration and time of exposure. Mortality results obtained are presented in tables 1 and 2. Table 1 depicts the lethality of the various concentrations of aqueous and ethanolic leaf extracts of *S. alata* to the mosquito species, while table 2 shows the lethality of aqueous and ethanolic stem extracts to the mosquito species.

At the end of the experiment (72h) the highest concentration of the aqueous leaf extract (0.75%w/v) resulted in mortality of 78.40, 55.20 and 41.60% for larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti* respectively. At the same concentration and exposure time the aqueous stem extract resulted in mortality of 60.80, 43.20 and 36.00% for larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti* respectively. Mortality values obtained when the vectors were exposed to the highest concentration of the ethanolic leaf extract (0.75% w/v) were 84.00, 69.60, and 50.40% for larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti* respectively. The highest concentration of the ethanolic stem extract (0.75%w/v) resulted in mortality of 63.20, 52.00 and 38.40% for larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti* respectively, at the end of experiment (72h).

The effects of the other concentrations of aqueous and ethanolic leaf and stem extracts of *S. alata* are also presented in tables 1 and 2. Extracts used for this investigation had different potencies as judged by their 72hLC_{50} values (table 3). The leaf extracts (ethanolic and aqueous) were more potent than the stem extracts. Mosquito species also demonstrated differential susceptibility to the extracts as revealed by the 72hLC_{50} values (table 3). For both the aqueous and ethanolic leaf extracts susceptibility was in the order:

An. gambiae > (0.480 & 0.503% w/v)	*Cu. quinquefasciatus* > (0.700 & 0.582% w/v)	*Ae. Aegypti* (0.840 & 0.791% w/v)

For the aqueous stem extract susceptibility was in the order:

An. gambiae > (0.628 % w/v)	*Ae. aegypti* > (0.935% w/v)	*Cu. quinquefasciatus* (0.989% w/v)

whereas susceptibility for the ethanolic stem extract was in the order:

Table 1: Percentage mortality of larvae of *An.gambiae, Cu.quinquefasciatus* and *Ae.aegypti* exposed to leaf extracts of *S. alata.*

Test organism/Exposure period										
Extraction solvent	Conc. % (w/v)	*An.gambiae*			*Cu.quinquefasciatus*			*Ae. aegypti*		
		24h	48h	72h	24h	48h	72h	24h	48h	72h
Ethanol	0.75	16.80	47.20	84.00	12.00	32.00	69.60	11.20	27.20	50.40
Aqueous	”	16.00	46.40	78.40	10.40	28.00	55.20	10.40	23.20	41.60
Ethanol	0.60	15.20	35.20	60.80	7.20	21.60	48.00	10.40	23.20	39.40
Aqueous	”	12.00	37.60	68.00	5.60	17.60	37.60	8.80	19.20	32.80
Ethanol	0.45	8.00	18.40	32.00	6.40	16.80	34.40	6.40	15.20	28.00
Aqueous	”	6.40	23.20	48.80	4.00	12.80	24.80	4.80	13.60	24.00
Ethanol	0.30	3.20	11.20	20.80	2.40	8.80	17.60	4.00	9.60	16.80
Aqueous	”	0.00	7.20	17.80	0.00	4.00	11.20	0.80	4.80	11.20
Ethanol	0.15	0.00	3.20	10.40	1.60	5.60	12.00	0.00	1.60	6.40
Aqueous	”	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2: Percentage mortality of larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti* exposed to stem extracts of *S. alata*

Test organism/Exposure period										
Extraction solvent	Conc. % (w/v)	*An. gambiae*			*Cu. quinquefasciatus*			*Ae. aegypti*		
		24h	48h	72h	24h	48h	72h	24h	48h	72h
Ethanol	0.75	15.20	35.20	63.20	11.20	28.80	52.00	7.20	20.80	38.40
Aqueous	”	14.40	33.60	60.80	8.80	22.40	43.20	7.20	20.00	36.00
Ethanol	0.60	10.40	24.00	42.40	8.00	20.00	40.80	5.60	16.00	30.40
Aqueous	”	10.40	24.00	44.00	7.20	17.60	33.60	4.80	13.60	24.00
Ethanol	0.45	5.60	15.20	27.20	6.40	17.60	35.20	0.80	6.40	16.80
Aqueous	”	5.60	14.40	32.00	5.60	13.60	24.80	0.00	4.00	11.20
Ethanol	0.30	1.60	5.60	14.40	4.80	14.40	28.80	0.00	4.00	11.20
Aqueous	”	3.20	8.80	15.20	3.20	8.80	16.00	0.00	0.00	4.00
Ethanol	0.15	0.00	0.00	0.00	0.00	4.00	13.60	0.00	0.00	0.00
Aqueous	”	0.00	0.00	0.00	0.00	2.40	7.20	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3: The 72hLC$_{50}$ values of aqueous and ethanolic extracts of *S. alata* Assayed against larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti.*

	Extract	*S.alata* /*An.gambiae*		Regression Equation	*S.alata/Cu. quinquefasciatus*		Regression Equation	*S.alata/Ae. aegypti*		Regression Equation
		72hLC$_{50}$ (%w/v)	95% conf. Interval (% w/v)		72hLC$_{50}$ (%w/v)	95% conf. interval (%w/v)		72hLC$_{50}$ (% w/v)	95% conf. Interval	
Aqueous	Leaf	0.480	0.449 - 0.513	Y=-2.434 + 4.638x	0.700	0.636 - 0.797	Y=-10.340 + 3.634 x	0.840	0.729 - 1.043	Y=- 8.634 + 2.952x
	Stem	0.628	0.576 - 0.701	Y=- 10.063 + 3.596x	0.989	0.786 - 1.470	Y=-5.572 + 1.860x	0.935	0.810 - 1.192	Y= - 11.033 + 3.714x
Ethanolic	Leaf	0.503	0.346 - 0.923	Y= - 8.110 + 3.002x	0.582	0.416 - 1.270	Y= - 6.785 + 1.533x	0.791	0.671 - 1.012	Y= - 6.403 + 2.209x
	Stem	0.635	0.584 - 0.706	Y= - 10.640 + 3.796x	0.761	0.615 - 1.087	Y= - 4.417 + 2.454x	0.923	0.785 - 1.199	Y= - 8.552 + 2.884x

An. gambiae > *Cu. quinquefasciatus* > *Ae. aegypti*
(0.635 % w/v) (0.761% w/v) (0.923% w/v)

Probit analysis using SPSS version 17 was employed in the determination of 72hLC$_{50}$ values.

The difference between the activity of aqueous and ethanolic extracts was determined using t-test at 95% level of significance. Softwares used were Microsoft excel and graph pad instat. The difference between the activity of aqueous and ethanolic extracts of *S. alata* assayed on all 3 mosquito species was not significant ($P > 0.05$).

DISCUSSION

The higher yields of aqueous extracts reported in this study than their ethanolic counterparts suggest that the plant parts used had higher proportion of water-soluble components. Generally, the yield of extracts was low compared to the amount of pulverized powder used for extraction. This is attributable to the method of extraction (maceration) employed. Maceration has generally been reported to give lower yield of plant extracts compared to soxhlet extraction (Ibrahim *et al.*, 1997), but it was preferred because it does not require heating, thus protecting thermolabile components.

Results obtained from the qualitative screening of the different parts of *S.alata* conoborate the reports of Owoyale *et al.* (2005), Abulude (2007) and El-Mahmood and Doughari (2008). Extracts used in this study tested positive to saponins. The larvicidal property of saponins has been reported by Wiesman and Chapagain (2006) and Pelah *et al.* (2002). Tannins were detected in the leaf and stem extracts of *S.alata*. Tannins are astringent (i.e. they can denature proteins). Khanna and Kannabiran (2007) reported that the larval mortality observed in their study could be attributed to the presence of saponins and tannins in the extracts they used. Alkaloids were detected in the leaf of *S. alata* and they have been shown in separate studies to be lethal to mosquito larvae (Francois *et al.*, 1996 and Lee, 2000). Flavonoids were detected in the leaf and stem of *S. alata*. Joseph *et al.* (2004) reported that flavonoids had larvicidal effect on *An. gambiae* and *Cu. quinquefasciatus*. Thus the larval mortality recorded in this study was attributed to the phytochemical compounds detected in the plant parts. This was substantiated by the fact that no mortality was recorded in the control experiments. Phytochemicals detected in the extracts used in this investigation may have exerted their effect on the larvae either singly or in synergy. Synergism as was demonstrated by the use of some plant extracts on *Ae. aegypti* and *Cu. annulirostris* has been documented by Shaalan *et al.* (2005).

The reduction in activity of larvae exposed to the different test solutions was as a result of the effect of the extracts because in the control set up all larvae were agile and activity wriggling throughout the duration of the experiment. Increase in mortality with increase in extract concentration and time of exposure reported in the study agrees with the reports of Obomanu *et al.* (2006), Rene *et al.*, (2006) and Khanna and Kannabiran (2007).

The differential potency of extracts reported in this study corroborates the report of Sukumar *et al.* (1991) 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. The observed difference in susceptibility of larvae to the extracts

reported in this research agrees with the reports of other researchers. For instance, Anyanwu and Uloko (2000) tested extracts of *Rothmania urcelliformes* (Rubiaceae) against mosquitolarvae. They reported that fourth instar larvae of *Ae. aegypti* were more susceptible to the extracts than fourth instar larvae of *Cu. quinquefasciatus*.

S. alata holds larvicidal potential and therefore warrants a more thorough exploitation. Work is presently going on to determine if fractions obtained from the crude extracts will demonstrate any improvements on the larvicidal activity. Further research work would reveal the active principle/s responsible for the observed larvicidal activity of the extracts of *S. alata.*

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