Bioactivity, Qualitative and Quantitative Components of *Alstonia Boonei* Leaf Extracts on Anopheles Mosquito Larvae in Nigeria

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***Abstract*—Malaria the most common disease in Africa is transmitted by the bites of female Anopheles mosquito. Different control measures have been applied to combat the spread of this infection including biological control measures. Our research focused on the use of water, ethanol and methanol extracts of a local medicinal plant; Alstonia boonei for 24 hours in vitro on different instars of mosquito larvae. The qualitative and quantitative components of these plants were also determined. The highest mortality was recorded in the ethanol treated larvae. Lethal concentration (LC) at LC50 and LC75 varied with the different extracts and instars. Phytochemical screening showed the presence of some chemicals that are insecticidal such as tannins, saponin and alkaloids. The quantitative components of these chemicals were also seen to vary significantly. Conclusively, our study revealed that the use of Alstonia boonei as vector control agents can reduce the spread of malaria infection.**

***Index Terms*—Mosquito, Malaria, Medicinal plant, Vector control agent.**

I. Introduction

Mosquitoes are the most important group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, lymphatic filariasis, dengue, Japanese encephalitis etc. resulting in millions of death every year (Das and Mukherjee, 2006). Malaria is endemic in most African countries and one of the major causes of sickness and death in sub-Saharan Africa, and it continues to be a major public-health challenge. It is possibly the most serious vector-borne disease worldwide. According to Kamalinder et al. (2008), the already-alarming number of deaths caused by malaria is increasing, caused in parts by the increase in mosquito resistance to chemical insecticides. Thus it is a huge challenge to mankind and puts great burden on the economies of especially third world countries (Breman et al., 2004)

In Nigeria, malaria is endemic and constitutes the major cause of death in children. Although it affects all ages, cases in children under the age of five are more likely to be serious, reflecting their relative low level of immunity to the disease compared with adults (Amodu et al., 2005). A major strategy of malaria control is to attack the vector with insecticides (Rajkumar and Jebanesan, 2009). Since the discovery of Dichloro-diphenyl-trichloroethane (DDT), mosquito control approach has been almost completely based on synthetic organic insecticides. However, the extensive use of synthetic organic insecticides during the last five decades have resulted in environmental pollution and also in the development of physiological resistance in major vector species in addition to the increased cost of insecticides. Hence, the need for search and development of environmentally safer, low cost and indigenous method of vector control.

Our research group focused on the locally available biodiversity resource in Nigeria. We assayed the larvicidal potency of a medicinal plant used locally for the treatment of malaria on the Anopheles arabiensis mosquito larvae; a major malaria vector in Nigeria.

II. Methodology

The selected plant was collected from different localities of Akure in Ondo State, one of the states in South West Nigeria (7o 10' 0" N and 5o 5' 0" E). The authentication of this plant was done at the Department of Forestry and Wood Technology of the Federal University of Technology Akure, Ondo State. The leaf samples were segregated, washed with tap water, shade dried at room temperature to retain their active ingredients. The dried samples were powdered in a table model grinder for extraction. The powdered materials were extracted using different solvents; water, ethanol and methanol. A 50g of the grinded samples was weighed into cleaned plastic containers and 150ml of each of the solvents was added and left for about 72 hours. The solvents along with extracts were drained out; filtered and semisolid extracts were obtained in vacuum using rotary evaporator. The semisolid extracts were lyophilized to obtain solid extracts

Assessment of larvicidal bioactivity of the plant extracts were conducted in the Microbiology Department of the Federal University of Technology Akure, Ondo State. The reared larvae used for the larvicidal bioassay were kept under laboratory conditions (27±20C and 80% humidity). Standard method for assaying larvicidal activity as recommended by World Health Organization (1996) was followed in all the assays. Twenty five larvae of second and fourth instars were released in 200ml of plastic containers for each set of experiment. Concentrations of 0.03, 0.05 and 0.10mg/mL were used and four replicates were used for each group of concentration. A negative control was also set up using an aqueous solution of 1% dimethylsulfoxide (DMSO). Mortality was recorded at the start of experiment

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(0 h), 12 h and 24 h of post treatments. Determination of the lethal concentration at LC50 and LC75 were done according to Finney Probit method, (1971). Phytochemical screening of the plant extracts was analyzed using standard methods (Harborne, 1973).

III. Results and Discussion

TABLE I: Phytochemical Screening of *A. Boonei*

Phytochemicals	Status
Saponin	+
Tannin	+
Phlobactannins	-
Alkaloid	+
Anthraquinone	-
flavonoid	-
Cardiac glycosides	
Keller killani's test	+
Salkowski Test	+
Lieberman's test	-
Legal Test	-

Key: present = +; not present = -

TABLE II: Quantitative Phytochemical Composition of *A. Boonei* from Water, Methanol and Ethanol Extracts.

Test	Water extract	Ethanol extract	Methanol extract
	A. boonei	*A. boonei*	*A. boonei*
Steroids (%)	1.2	1.7	0.6
Tannins (mg/ml)	0.1	0.1	0.1
Phlobatanins (%)	0.8	1.6	1.7
Alkaloids (%)	1.8	3.1	2.8
Saponins (mg/ml)	1.8	1.7	1.1
Flavonoids (mg/ml)	1.2	1.2	1.2
Phenols (mg/ml)	0.2	1.0	0.5
Cardiacglycosides (%)	0.2	1.1	1.1

TABLE III: Larvicidal Activity of *A. Boonei* on 2ND Instar *A. Arabiensis* Larvae at 12 Hours after Treatment

Concentration (mg/ml)	Mortality [a, b] (%) ±SE		
	Water extract	Methanol extract	Ethanol extract
0.03	10.23^{a}±0.57	23.00^{a}±1.42	41.35^{a}±0.57
0.05	12.67^{b}±1.15	38.67^{b}±1.15	53.67^{b}±1.52
0.10	27.66^{c}±1.15	55.00±^{c}2.00	56.33^{b}±2.50

There were four replicates per treatment with 25 larvae per replicate. Mean with columns with the same letters are not significantly different P ≤0.05

TABLE IV: Larvicidal Activity of *A. Boonei* on 2ND Instar *A. Arabiensis* Larvae at 24 Hours after Treatment

Concentration (mg/ml)	Mortality [a, b] (%) ±SE		
	Water extract	Methanol extract	Ethanol extract
0.03	18.00^{a}±1.00	32.72^{a}±2.88	53.33^{a}±2.52
0.05	26.00^{b}±1.70	63.58^{b}±2.51	73.66^{b}±2.52
0.10	25.30^{b}±2.52	87.00^{c}±2.00	98.33^{c}±2.88

There were four replicates per treatment with 25 larvae per replicate. Mean with columns with the same letters are not significantly different P ≤0.05

TABLE V: Larvicidal Activity of *A. Boonei* on 4TH Instar *A. Arabiensis* Larvae at 12 Hours after Treatment

Concentration (mg/ml)	Mortality [a, b] (%) ±SE		
	Water extract	Methanol extract	Ethanol extract
0.03	11.33^{a}±1.15	42.33^{a}±2.52	44.00^{a}±1.00
0.05	18.66^{b}±0.57	43.33^{a}±1.53	52.00^{b}±2.64
0.10	23.33^{c}±1.52	54.67^{b}±2.08	56.33^{c}±1.52

There were four replicates per treatment with 25 larvae per replicate. Mean with columns with the same letters are not significantly different P ≤0.05

TABLE VI: Larvicidal Activity of *A. Boonei* on 4TH Instar *A. Araebiensis* Larvae at 24 Hours after Treatment

Concentration (mg/ml)	Mortality [a, b] (%) ± SE		
	Water extract	Methanol extract	Ethanol extract
0.03	14.37^{a}±1.52	64.00^{a}±2.64	71.30^{a}±2.89
0.05	16.67^{a}±1.15	72.00^{b}±3.24	75.33^{b}±1.07
0.10	27.00^{b}±2.00	71.66^{c}±2.45	82.00^{c}±0.15

There were four replicates per treatment with 25 larvae per replicate. Mean with columns with the same letters are not significantly different P ≤0.05

TABLE VII: Relative Potency of *A. Boonei* Extracts on Different Instars of *Anopheles* Larvae.

AGE	TIME (HRS)	Water extract		Methanol extract		Ethanol extract	
		LC50	LC75	LC50	LC75	LC50	LC75
2ND INSTAR	12	12.40	48.08	2.46	14.94	2.32	6.17
	24	38.55	62.49	44.5	16.04	1.42	3.14
4TH INSTAR	12	15.56	72.39	7.32	34.49	4.23	18.33
	24	11.33	51.95	12.54	6.24	2.70	4.14

The qualitative and quantitative screening of A. *boonei* revealed the presence of some compounds at varying percentages (Table I and II). These compounds include saponin, tannin, alkaloid and flavonoid, which might have contributed to the larvicidal potency displayed in this study. This agreed favourably with the report of Isman (1997), who reported that natural defense of plant against insects consist almost mixtures of closely related compounds, rather than a single toxicant. Tables IV, V, VI and VII showed that the percentage mortality of *Anopheles* mosquito larvae in all the treatment varied greatly with the age of the larvae, concentrations of the plant extracts and the time of exposure of the larvae of the extracts. The plants were more effective at high concentrations, the toxic effect however increased with increase in the concentrations of the extracts. A moderate effect of A. *boonei* at lower concentration was observed but exhibited higher activity as the concentration increases. At 0.03mg/mL it caused 41.35 and 23% in ethanol and methanol extracts respectively while 53.67 % and 38%; 55 % and 56.33%, mortality respectively at 0.05mg/mL and 0.10mg/mL concentrations in the second instar larvae at 12 h post treatment. A general observation of low mortality percentage was recorded in water extracts

treatments. These variations in susceptibility of extracts to mosquito's larvae may be due to variations in extracting solvents, mosquito species or exposure periods (Umar, 2002). The results from this present study also confirmed the previous works of using plants as insecticides. Fernando (2005) reported that most plants are known to possess chemicals substances like terpenoides, saponins, tannins, flavonoids and alkaloids among others which have found to have reasonable efficacy against a range of mosquito species. According to Table II of our result higher percentage of alkaloids was observed; 1.8, 2.8 and 3.1% at water, methanol and ethanol extracts respectively. This might be as a result of the plant constituent that have toxic effect on the mosquito larvae. This observation was further confirmed by Chakkaravarthy *et al*., (2011) who reported that biological activity of some plants extracts may be due to various compounds including flavonoids and alkaloid existing in the plants.

IV. Conclusion

Our finding in this study has shown that *A. boonei* leaf extracts have a reasonably high efficacy against *Anopheles* mosquito larvae. Hence it can be suggested as a natural larvicidal agent for controlling this species of insect that has constituted nuisance to our community and a public health challenge in this present generation.

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