LARVICIDAL EFFECTS OF LEAF, BARK AND NUTSHELL OF *Anacardium occidentale* ON THE LARVAE OF *Anopheles gambiae* IN EBONYI STATE, NIGERIA

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ABSTRACT

Comparative analysis of the larvicidal properties of aqueous extracts of leaves, bark and nutshell of Anacardium occidentale L. (Cashew) were evaluated on the larvae of Anopheles gambiae. Three concentrations of 10/100ml, 20/100ml and 30/100ml each of leaf, bark and nutshell were prepared in three replicates. The treatments were exposed to two durations of thirty minutes and one hour. After thirty minutes of treatment, the mean mortalities were 53%, 68% and 56% for leaf, 64%, 71% and 57% for bark and 53%, 68% and 58% for nutshell, while the mean mortalities after one hour of exposure were 68%, 70% and 93% for leaf, 84%, 93% and 97% for bark and 61%, 68% and 73% for nutshell. Analysis of variance (ANOVA) showed that at thirty minutes of exposure, there were no significant difference (P<0.05) between concentrations and the percentage mortality, but it was highly significant (P<0.01) after 1 hour of exposure. Quantities phytochemical analysis revealed the presence of tannin, oxalate, stearic acid, glucoronic acid and glutamic acid in the leaf, bark and nutshell extracts.

Keywords: Larvicidal, *Anacardium occidentale* extracts, Phytochemical analysis, *Anopheles* larvae mortality

INTRODUCTION

Malaria is one of the world's most important infectious diseases and is responsible for great morbidity and mortality. There are estimated rates of about 300 million new infections every year world wide, that results in 1 – 2 million deaths (Deribe, 2008). Mosquitoes are the principal vectors of malaria and other dipteran vector borne diseases (Kaushik and Saini, 2008). Different strategies have been devised to reduce the prevalence of malaria and other mosquito-borne diseases in the endemic regions of the world, particularly in Sub-Sahara Africa. Biological control at the larval stages of development of mosquitoes is one of those techniques, which afford cheap, easy to use and environmental friendly methods of malaria control (Obomanu *et al*., 2006).

Consequently, interest in plant-based products has been revived because of the development of resistance, cross-resistance, possible toxicity hazards associated with synthetic insecticides and their rising cost. The phytochemical compounds obtained from the huge diversity of plants species from the tropical forests are important sources of safe and biodegradable chemicals, which can be

screened for larvicidal activities (Ansari *et al.,* 2000). A large number of plant extracts have been reported to have excellent larvicidal activities, such plants as *Azadirachta indica, Cymbopogan* sp*, Eucalyptus sp, Maculata citriodon* (Mittal and Subbarao, 2003), *Draceana aborea* and *Vitex doniana* (Nnamani *et al*., 2007), *Hyptis suaveolens* (Ivoke *et al*., 2009) among others.

A growing need to search for more plants with larvicidal effects from the rich flora biodiversity of the country, has led to the present investigation where the plant, *A. occidentale* belonging to the family Anacardiaceae, commonly known as cashew or cajueiro was studied. The family contains 73 genera and about 600 species with the genus *Anacardium* having about 8 species (Eijnatten, 1985). They are distributed around the moist warm temperate to tropical very dry and wet-forest zones of the world (Johnson, 1993).

The bark and leaves of cashew contain an anti-inflammatory and astringent agent, effective in the treatment of diarrhea, diabetes, eczema, and also used as mouth wash (Akinpelu, 2001). It is also used in leather tanning, as a catalyst in the treatment of premature aging of the skin and skin rematerialization because it contain volatile compounds like esters, trepans and carboxylic acid (Kubo *et al.,* 1999). The resin found in cashew is also used as an expectorant, cough remedy and insect repellent. The leaves and barks are brewed and taken as herbal tea to treat diarrhea, colic remedy in infants, antiseptic vaginal douche and an astringent to stop bleeding after tooth extraction. The toxic seed oil in the nut is used as an external worm medicine to kill botfly larvae under the skin. They are also used in the manufacture of adhesives, resins and natural insecticides (Nayar, 1995).

The aims of this research work were: to determine the bioactive compounds contained in the leaf, bark and nutshell *A. occidentale,* to assay the larvicidal effects of different aqueous extracts of the leaf, bark and nutshell of *A. occidentale* on the larvae of *An. gambiae*, and to compare mortalities of different aqueous extract concentrations at 30 minutes and one hour time intervals.

MATERIALS AND METHODS

Mosquito Larvae: *Anopheles gambiae* larvae were obtained from mosquitoes ovipositing sites found indoors at Student's Hostel, Ebonyi State University, Abakaliki, Nigeria, in July 2008. They were taxonomically identified (Medler, 1980) and confirmed as *An. gambiae* larvae by an Entomologist in the Department of Zoology, University of Nigeria, where voucher specimens (AnGL - 011) were deposited in their Museum of Natural History. The female *An. gambiae* were provided with the opportunity to feed on blood of sheaved albino rat that was kept in a cage covered with mosquito net. The colony of the larvae was kept at ambient temperature in a plastic bowl, half filled with pond water so that the larvae will feed on the microorganisms.

Plant Extract Preparation: The *A. occidentale* leaves, bark and nutshell were collected from Mbukobe clan in Ebonyi State. The plant was taxonomically identified (Keay, 1965) and confirmed as *A. occidentale* L. by a curator in Botany Department, University of Nigeria, where voucher specimen (Ao - 019) was deposited in the herbarium. The leaves and the stem barks were oven dried at 70^0C for 60 minutes, while the nutshells were oven dried at 70^0C for 45 minutes because of its high oil content. The dried leaves, bark and nutshells were ground using pestle and mortar. Twenty grams of the pulverized leaves, bark and nutshell were introduced into 200 ml of distilled water in a plastic container with cover and allowed to stand for 24 hours. The soaked pulverized leaves, bark and nutshell were filtered with Whatman Filter Paper No. 1 into three glass beakers respectively to get the extracts.

Phytochemical Screening: Phytochemical screening of the leaves, bark and nutshells were carried using standard quantitative method (Farnsworth, 1966) at Crop Science Department, University of Nigeria, Nsukka.

Table 1: Phytochemical compositions of leaf, bark and nutshell of *Anacardium occidentale*

A. occidentale	Tannin (mg/kg)	Oxalate (mg/kg)	Stearic acid (mg/kg)	Glucuronic acid (mg/kg)	Glutamic acid (mg/kg)
Leaf	0.9	1.3	4.3	3.5	2.2
Bark	0.7	0.6	2.8	2.3	1.5
Nutshell	0.3	0.6	2.8	2.3	1.5

Table 2: Mean mortality of larvae of *Anopheles gambiae* exposed to different treatments of leaf, bark and nutshell extracts of *Anacardium occidentale* for thirty minutes

Conc. of extract (ml)		Leaf	Bark		Nutshell	
10/100		53.33 ± 0.07	64.44 ± 0.02		53.33 ± 0.07	
20/100		68.89 ± 0.02	71.11 ± 0.02		68.89 ± 0.02	
30/100		56.67 ± 0.04	57.78 ± 0.02		58.89 ± 0.06	
Source of variations	**df**	**Sum of squares**	**Mean of squares**	**f-calculated**	**f- tab 5%**	**f- tab 1%**
Treatment SS	2	40.3678	80.7356	1.3947	5.14	10.92
Error SS	6	347.3261	57.8877			
Total SS	8	387.6939				

Larvicidal Bioassay: The larvicidal activities were evaluated by subjecting the *An. gambiae* larvae to the same concentrations of extracts (leaves, bark and nutshell) of 10 ml each in Petri-dishes containing 100 ml of water respectively. Three replicates of the above were made. Newly molted third and fourth instars larvae of 30 each were introduced into the concentrations. The percentage larval mortalities were recorded after thirty minutes and one hour intervals, respectively. Dead larvae were identified when they failed to move after probing with a needle. Dead larvae in the three replicates were combined and expressed as mean percentage of larvae mortality. Analysis of variance (ANOVA) was done using the mean of the total replications, to determine the significant effects using student t-test.

RESULTS AND DISCUSSION

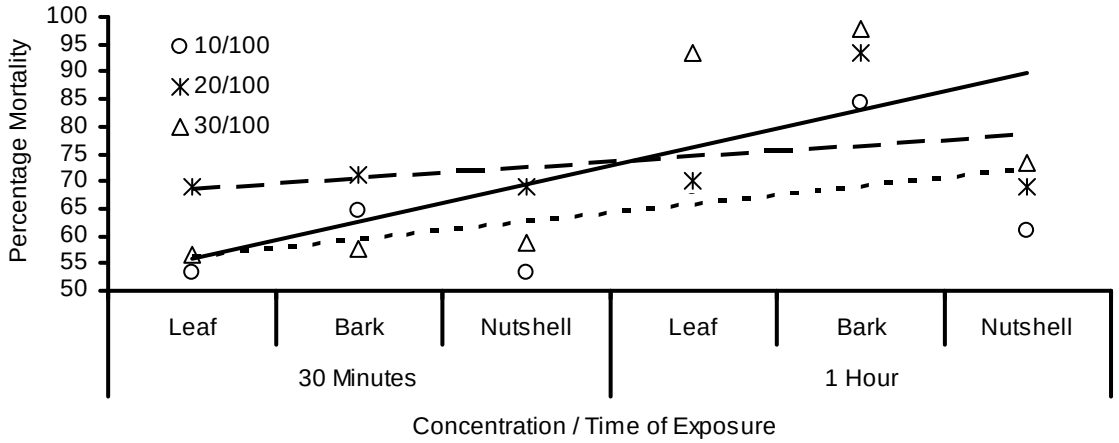
Phytochemical analyses of *A. occidentale* indicated the leaf, bark and nutshell were richer in stearic acid than other measured phytochemicals (Table 1). Other phytochemicals present were of tannin, oxalate, glutamic and glucuronic acids. Stearic acid had the highest value of (4.3 mg/kg) in the leaf, while tannin in nut shell had the lowest value of (0.3 mg/kg). However, the leaf has the highest content of these chemicals but exerted less effect on the test organisms, when compared with the bark that had lower content of those chemicals, but exerted higher larvicidal effect on the test organisms. This finding was in agreement with the report of Sukumar *et al.* (1991). They established that the activity of phytochemicals on targeted species varies with respect to plant parts from which they were extracted; solvent of extraction, geographical origin of the plant and photosensitivity of some of the compounds in the extract.

The larvicidal activities of aqueous extracts of Leaf, Bark and Nutshell of *A. occidentale* showed that the plant extracts have insecticidal properties on the targeted organisms (Tables 2 and 3). Mortality of *An. gambiae* larvae exposed to the plant extracts increased with time of exposure and concentration of extracts as was also reported for larvae of *Cu. quinquefasciatus* exposed to extracts of *Nerium indicum* and *Euphorbia royleana* (Srivastava *et al.,* 2003; Choochote *et al.,* 2003).

Highest mortality was recorded for 30 ml of all the extracts with 68% for leaf, 71% for bark and 68% for nutshell at thirty minutes (Table 2), while 97% mortality for bark, 93% mortality for leaf and 73% mortality for nutshell (Table 3) were recorded at one hour intervals.

Table 3: Mean percentage mortality of *Anopheles gambiae* larvae exposed to different treatments of Leaf, Bark and Nutshell extracts of *Anacardium occidentale* for one hour

Conc. of extract (ml)		Leaf	Bark		Nutshell	
10/100		68.89 ± 0.02	84.44 ± 0.02		61.11 ± 0.02	
20/100		70.00 ± 0.10	93.33 ± 0.07		68.89 ± 0.02	
30/100		93.33 ± 0.07	97.78 ± 0.03		73.34 ± 0.09	
Source of variation	**df**	**Sum of squares**	**Mean of squares**	**f-calculated**	**f- tab 5%**	**f- tab 1%**
Treatment SS	2	880.6475	1761.295	19.2196*	5.14	10.92
Error SS	6	549.8432	91.6405**			
Total SS	8	1430.4907				



% mortality of larvae = 3.2703(10/100 ml extract) + 52.811 R^2 = 0.2762

% mortality of larvae = 1.9363(20/100 ml extract) + 66.741 R^2 = 0.1381

% mortality of larvae = 6.794(30/100 ml extract) + 49.186 R^2 = 0.4683

Figure 1: Increased mortality of *Anopheles gambiae* larvae exposed to *Anacardium occidentale* extracts with time of exposure and the concentration of extracts

This was in line with the work of Kaushik and Saini (2008) which reported that acetone extracts of *Millingtoning hortensis* had very high larvicidal activity, irrespective of the larvae species of mosquitoes tested. The extracts killed 98.33% of the larvae at 300 ppm. Earlier reports have demonstrated that several plant species possess insecticidal properties under laboratory conditions e.g. *Azadirachta indica, Cymbopogan* sp*, Eucalyptus sp, Maculata citriodon* (Mittal and Subbarao, 2003), *Draceana aborea* and *Vitex doniana* (Nnamani *et al*., 2007) and *Hyptis suaveolens* (Ivoke *et al*., 2009). The result also revealed that mortality of *An. gambiae* larvae exposed to the plant extracts increased with time of exposure and the concentration of extracts (Figure 1). The effects of the extracts was reported to be dose dependent as evident by increased in the percentage mortality with increasing concentrations at one hour duration. This result was in conformity with the works of Srivastava *et al.* (2003) and Choochote *et al.* (2004) which reported that *larvae* of *Culex quinquefasciatus* exposed to extracts of *Nerium indicum* and *Euphorbia royleana* showed high mortality with increased time of exposure and concentration of extracts.

In the present result, differences on the mortality of different larval stages of the *Anopheles gambiae* revealed that the third instars were more susceptible to the extracts at thirty minutes and one hour than the fourth larval instars which showed less susceptible to the extracts at thirty minutes but higher susceptibility to the extracts at one hour time

exposure. Similar observations were reported by *Al-Sharook et al.* (1991) for larval *Culex* spp. exposed to crude extracts of Melia *volkensii and M. azaderach* respectively, and Ivoke *et al.* (2009) for *An. gambiae larvae* exposed to *H. suaveolens* aqueous extract.

The analysis of variance (ANOVA) between the mortality rate of the larvae and time intervals showed that there was no significant difference ($P > 0.05$) at thirty minutes in larvae mortality while there was significant difference ($P < 0.05$) at hourly duration in larvae mortality. This was contrary to the result of Obomanu *et al.* (2006), which indicated that the cumulative mortality of *Cu. quinquefasciatus* and *An. gambiae* exposed to *L. alopecuroides* and Neem were significantly different ($P < 0.05$) at the various concentrations and time of exposures.

Conclusion: From this study, it could be said that *A. occidentale* has potential bioactive compounds against larvae of *Anopheles gambiae*. The extracts from the plant could be used in stagnant water bodies, which are known to be breeding ground for mosquitoes. However, further research on the bioactive compounds found on the leaf and bark of the plant are highly recommended. More research work should be done on the phytotoxicity of *A. occidentale.*

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