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Control of Mosquito Larva Using Bark Extracts of *Gmelina arborea*

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Abstract

The application of plant derived pesticides for the control of vectors and pathogen have become global. Notwithstanding, synthetic therapies have been most applied, but it poses some ecotoxic problem when misapplied. The biocidal activities of 3 solvent (chloroform, Methanol and Ethanol), bark extracts of *Gmelina arborea* was investigated against vectors of malaria (*Anopheles Gambiae*). Results show that the chloroform extract has LC_{50} value of 4.90 ppm. Furthermore, the ethanol and methanolic extracts had LC_{50} values of 4.00 and 2.20 ppm respectively. Therefore, the order of activities of the bark extracts of *G. arborea* were chloroform>ethanol>methanol. Based on results of this study, we therefore recommend the plant for the formulation of pesticide for the control of malaria and vector-borne diseases.

Keywords: *Anopheles Gambiae*, *Gmelina arborea*, Bark extract, Solvents

1 Introduction

Malaria is a tropical and rampant vector-borne diseases transmitted by female anopheles' mosquito [1, 2]. Records of the WHO as documented in literature indicated that malaria ranks first amongst vector borne diseases, with significant global morbidity and mortality burden [3]. In Africa Malaria is endemic in over 40 countries, including Nigeria with over 500 million of the population at risk [4], especially children and pregnant women [5].

Gmelina arborea is a deciduous and eco-tolerant plant which belonging to the *verbenaceae* family. It is endemic in several continents, and thrives in vast and extreme weather conditions, especially in the tropics and Asia [6]. The therapeutic applications of the plant have already documented in literature. It generally has antibacterial and antidiabetic and antioxidant properties [7]. The root and bark extracts were effective as laxative, and also used to relief stomach ache and piles [8, 9].

Furthermore, the multifaceted nature of the *G. arborea* have been documented in literature including its; anti-vernominal properties, anti-schistosomal properties [10]. Also, antioxidant properties [11]. Notwithstanding, there are several challenges encountered in the fight against vector-borne disease like malaria.

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They include but not limited to the ecotoxicity of synthetic pesticides [2], re-infection after drug administration [12], as well as the rapid prolificacy of the vectors [13]. The application of synthetic pesticides against vectors that transmit diseases is not far-fetched. On the other hand, the problem associated with wrong use of synthetic pesticides can be toxic and adverse to other species [2, 3].

2 Materials and Methods

2.1 Collection and preparation of plant Extract

The Bark of *G. arborea* was collected along Swali Market road in Yenagoa Local Government Area of Bayelsa State, Nigeria. Three hundred grams (300 g) of fresh bark of the plant was weighed (Satoric AG Gottingen Electronic weighing balance). The weighed bark was chopped into tiny bits and pounded using clean ceramic mortar and pestle. It was then macerated in 500 ml of the respective solvents being; Chloroform, Ethanol and Methanol (BHD Chemical Ltd. Poole England) for 72 hours. Afterwards, it was filtered into a clean and sterile conical flask using whatman no.1 filter paper [14]. The filtrates of the macerated concoctions were respectively extracted using a rotary evaporator (60°C). The obtained extracts (i.e. extracted active ingredients) were allowed to cool and preserved for the bioassay at low temperature (4°C).

2.2 Vector Collection/Breeding of Larvae

Mosquito Larvae belonging to the genus *Anopheles* (*An. gambiae*), was used for the study. The larvae were cultured in the wild using baits positioned around conspicuous breeding sites. Plastic containers and automobile tyres half-filled with water, and sand was used as the breeding bait in conspicuous breeding site. The baits were constantly monitored for the conspicuous emergence of mosquito larvae. Prior to the bioassay, the bred larvae were placed on an enamel tray with dechlorinated water (pH 7.4), and acclimatized to laboratory condition [15, 16].

2.3 Experimental Set Up

For the purpose of the bioassay, samples of 20 larvae and snails, were distinctly placed in a 500ml solution of the extracts, in a 24-hour static non-renewal test. It was performed in accordance with the World Health Organization guidelines [17]. The mortality rates of organisms were observed and recorded. Dipex pesticide was used as the positive control, while 500ml of distilled water adjusted with 2.5 ml of 10% dimethyl sulfoxide (DMSO) at pH 7.5, was used as the negative control [12, 15].

2.4 Biolarvicidal Screening Test

In a rapid screening test, triplicate concentrations of 50 - 10ppm were used to screen the larva and snails for total (i.e. 100%) mortality within 24 hours in order to detect the range of activity. The replicates of the extracts which demonstrated total average mortality (i.e. 100% mortality) on larva at 10ppm during the rapid screening. The screening was carried out at different concentrations, in order to determine the minimal total lethal concentrations (LC_{100}).

2.5 Statistical Analysis

The data for mortality rates were expressed as mean± standard deviation using version 20 of SPSS. A one-way analysis of variance was used to carry out the statistical analysis, while Duncan multiple range test was used to determine the source of observed difference using SPSS Version 20. Furthermore, the median Lethal doses (LC_{50}) of the seed sap against the larva, were estimated from the average minimal lethal concentrations in a concentration-mortality using Microsoft 2016 excel package.

3 Results and Discussion

The mortality rates of all solvent bark extracts including Chloroform, Methanol and Ethanol, assayed against the larvae of *An. gambiae* are presented in Tables 1, 2 and 3. For the biolarvicidal bioassay, the positive control induced total mortality at 1.00ppm, as opposed to the negative control had no lethal or sublethal effects against all larvicidal bioassay (Tables 1 -3). However, the solvent extracts demonstrated varying degrees of mortalities at concentrations ranging from 1.00 - 10.00 ppm ($p<0.05$). As presented in Table 1, for the Chloroform extract bioassay, mortality rate increases with corresponding increase in concentration. Statistically there was significant difference ($p<0.05$) at all concentrations, with minimal lethal concentration at 10.00 ppm (Table 1).

Table 2 presents the mortality rates for An. gambiae screened against methanolic bark extract of *G. arborea*. Results of the bioassay similarly indicated that the positive control induced total mortality at 1.00ppm, whereas the negative control demonstrated no mortality against the larvae (Table 2). Furthermore, while the varying degrees of mortalities increased with concentration with significant difference ($p<0.05$), the minimal lethal concentration was demonstrated at 7.00ppm.

Table 3 presents the mortality rates of ethanol bark extract of G. arborea screened against larva of *An. gambiae*. In a similar fashion, while the negative control had no effect on the larvae, the positive control was lethal at 1 ppm. Mortality rate with increase in concentration, with minimal lethal concentration at 9.00ppm. There were significant differences amongst the various concentration, except for the minimal lethal concentration.

The activities of the solvent extracts were assayed against the larvae in a concentration-mortality curve as presented in Figure 1. The Chloroform extract had LC_{50} value of 4.90 ppm, a more active LC_{50} value of 4.00ppm was exhibited by the ethanol bark extract. Meanwhile, the highest activity was demonstrated by the Methanolic bark extract with LC_{50} value of 2.20ppm. While all extract of *G. arborea* exhibited biolarvicidal activities. The larvicidal activities are as a result of diverse bioavailable phytochemicals in the plant, phytochemicals like; saponin, alkaloid, tannin, phenol, flavonoid and the variation in activities of plant extracts are influenced by the various applied solvents [2, 18]. In another study phytochemical like; methyl arboreal, arboreal, isoarboreol, glummadiol, flavonoid, alkaloids, gmelanone, n-hexacosnol, sitostereol and hutteolin have been isolated from *G. arborea* [19].

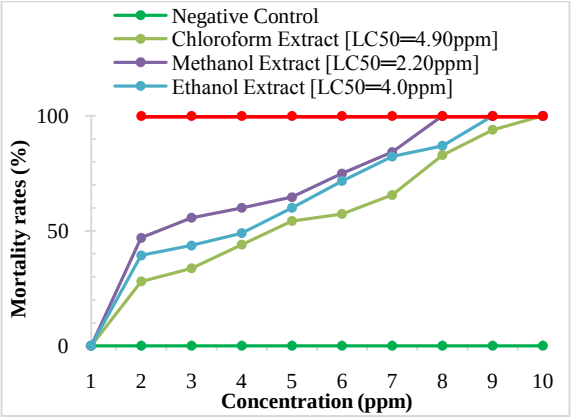

Figure 1: Concentration-mortality of express seed sap extracts of *G. arborea* against *An. gambiae*

A recent study showed that the express seed sap extracts of *G. arborea* against *An. gambiae* was lethal with LC50 values of 2.25 ppm; as well as vectors of schistosomiasis being *Bulinus globosus* and *B. pfeifferi* with LC50 values of 0.75 and 8.00ppm respectively (Angaye et al., 2017b). In another study, using several solvents (crude, methanol, ethanol, chloroform, hexane) extracts of G arborea demonstrated significant mortality rates (Angaye et al., 2017a).

Table 1: Mortality rates for *An. gambiae* Chloroform Extract Larvicidal Bioassay

Concentration (ppm)	Mortality Rates (%) Mean±SD			95% Confidence Interval for Mean		Minimum	Maximum
	Chloroform Extract	Positive Control	Negative Control	Lower Bound	Upper Bound		
0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.0000	0.0000	0.00	0.00
1.00	21.00±2.00b	100.00±0.00k	0.00±0.00a	16.0317	25.9683	19.00	23.00
2.00	28.00±3.00c	100.00±0.00k	0.00±0.00a	20.5476	35.4524	25.00	31.00
3.00	33.67±3.00d	100.00±0.00k	0.00±0.00a	26.0775	41.2558	31.00	37.00
4.00	44.00±3.00e	100.00±0.00k	0.00±0.00a	36.5476	51.4524	41.00	47.00
5.00	54.33±3.05f	100.00±0.00k	0.00±0.00a	46.7442	61.9225	51.00	57.00
6.00	57.33±3.06g	100.00±0.00k	0.00±0.00a	49.7442	64.9225	54.00	60.00
7.00	65.67±3.05h	100.00±0.00k	0.00±0.00a	58.0775	73.2558	63.00	69.00
8.00	83.00±4.58i	100.00±0.00k	0.00±0.00a	71.6163	94.3837	78.00	87.00
9.00	94.00±3.00j	100.00±0.00k	0.00±0.00a	86.5476	101.4524	91.00	97.00
10.00	100.00±0.00k	100.00±0.00k	0.00±0.00a	100.0000	100.0000	100.00	100.00

Table 2: Mortality rates for *An. gambiae* Methanolic Extract Larvicidal Bioassay

Concentration (ppm)	Mortality Rates (%) Mean±SD			95% Confidence Interval for Mean		Minimum	Maximum
	Methanol Extract	Positive Control	Negative Control	Lower Bound	Upper Bound		
0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.0000	0.0000	0.00	0.00
1.00	47.00±2.00a	100.00±0.00g	0.00±0.00a	42.0317	51.9683	45.00	49.00
2.00	55.67±1.55b	100.00±0.00g	0.00±0.00a	51.8721	59.4612	54.00	57.00
3.00	60.00±1.00c	100.00±0.00g	0.00±0.00a	57.5159	62.4841	59.00	61.00
4.00	64.67±2.52cd	100.00±0.00g	0.00±0.00a	58.4151	70.9183	62.00	67.00
5.00	74.67±4.04d	100.00±0.00g	0.00±0.00a	64.6271	84.7062	71.00	79.00
6.00	84.33±2.08e	100.00±0.00g	0.00±0.00a	77.1622	87.5045	80.00	84.00
7.00	100.00±4.00f	100.00±0.00g	0.00±0.00a	77.0634	96.9366	83.00	91.00
8.00	100.00±0.00f	100.00±0.00g	0.00±0.00a	100.0000	100.0000	100.00	100.00
9.00	100.00±0.00f	100.00±0.00g	0.00±0.00a	100.0000	100.0000	100.00	100.00
10.00	100.00±0.00f	100.00±0.00g	0.00±0.00a	100.0000	100.0000	100.00	100.00

Table 3: Mortality rates for *An. gambiae* Ethanolic Extract Larvicidal Bioassay

Concentration (ppm)	Mortality Rates (%) Mean±SD			95% Confidence Interval for Mean		Minimum	Maximum
	Ethanol Extract	Positive Control	Negative Control	Lower Bound	Upper Bound		
0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.0000	0.0000	0.00	0.00
1.00	34.67±1.53b	100.00±0.00h	0.00±0.00a	30.8721	38.4612	33.00	36.00
2.00	39.33±1.52c	100.00±0.00h	0.00±0.00a	35.5388	43.1279	38.00	41.00
3.00	43.67±1.53cd	100.00±0.00h	0.00±0.00a	39.8721	47.4612	42.00	45.00
4.00	49.00±2.00d	100.00±0.00h	0.00±0.00a	44.0317	53.9683	47.00	51.00
5.00	60.00±3.61e	100.00±0.00h	0.00±0.00a	51.0433	68.9567	56.00	63.00
6.00	71.67±2.65f	100.00±0.00h	0.00±0.00a	68.4276	81.5724	73.00	78.00
7.50	82.33±2.52g	100.00±0.00h	0.00±0.00a	78.0817	90.5849	82.00	87.00
8.00	87.00±3.11h	100.00±0.00h	0.00±0.00a	100.0000	100.0000	100.00	100.00
9.00	100.00±0.00i	100.00±0.00h	0.00±0.00a	100.0000	100.0000	100.00	100.00
10.00	100.00±0.00i	100.00±0.00h	0.00±0.00a	100.0000	100.0000	100.00	100.00

These phytochemicals which were reported to support the bioactivities of the plant include; flavonoid, alkaloids, arboreal, isoarboreol, methyl arboreal, glummadiol, gmelanone, n-hexacosnol, sitostereol and hutteolin. The antimicrobial activities of *G. arborea* was also reported against some pathogenic microbes (Kaswale *et al*., 2012; Ishaku *et al*., 2012).

4 Conclusion

Three solvent bark extracts of *G. arborea* were investigated against mosquito larvae. Fortunately, all solvent extract shows promising larvicidal activities against the larvaes with the methanolic extract having the highest activity. Based on results of this study we therefore recommended *G. arborea* as a potential candidate for alternative formulation of pesticide for the control of malaria. In addition, we also recommend further study for field application of this plant.

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