



CONTROL OF MALARIA AND SCHISTOSOMIASIS VECTORS USING EXPRESS SEED SAP EXTRACTS OF *GMELINA ARBOREA*

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ABSTRACT

Researches on plant derived pesticides for their control against vectors that transmit major diseases of public health have become a global mantra. Although the application of synthetic therapies is remarkable, but unfortunately synthetic pesticides pose serious eco-friendly challenge, while drug administration can only abate morbidity burden and reinfection frequency. The biocidal activities of fresh express seed sap extracts of *Gmelina arborea* was investigated against vectors of malaria (*Anopheles gambiae*) and schistosomiasis (*Bullinus globosus* and *Biomphalaria pfeifferi*). Results show that the *An. gambiae* bioassay has LC50 value of 2.25 ppm. Furthermore, the *G. arborea* extract against *B. globosus* had LC50 value of 0.75 ppm compared to the *B. pfeifferi* which was less susceptible with LC50 value of 8.00ppm. Notwithstanding, all assayed extracts were effective against the vectors. As such, based on the finding of this study, *G. arborea* is therefore recommended as a candidate amongst several bioactive agents for the control of vector-borne diseases.

Keywords: Pesticides, Mosquito, eco-friendly, Larvicides, Molluscicides

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1. INTRODUCTION

Malaria and Schistosomiasis are vector-borne diseases transmitted by female anopheles mosquito and parasite of the genus schistosoma. Schistosomiasis have intermediate host, which are minute aquatic snail belonging to the genera; *Biomphalaria*, *Bulinus* and *Oncomelia* [1, 2]. Statistically, it is documented in literature that malaria and schistosomiasis ranks first and second amongst vector borne diseases, in terms of their global morbidity and mortality burden [3]. Schistosomiasis was reported to affect about 4 - 5% of the world population [4], which makes up about 200 to 207 million morbidity burden [5 - 7], with about 20 million and 120 million of acute and chronic cases respectively [5, 8]. Schistosomiasis affects human, as well as wild and domestic animals [9, 10].

The disease is endemic in continents like; Africa, Caribbean, Asia, Middle East and South America. Furthermore, schistosomiasis is endemic in over 70 countries, with about 200 to 207 million cases [5]. Malaria is prevalent in more than 100 countries, with an annual morbidity burden of over 700 million [11]. Schistosomiasis can affect both Human as well as some wild and animals depending on the specie of the intermediate host (snail). For instance, human intestinal schistosomiasis caused by *Schistosoma mansoni* whose intermediate host belongs to the *Biomphalaria* genera (*B. glabrata*, *B. alexandrina*, *B. pfeifferi*). Also, the intermediate host for this study belongs to the *Bulinus* genus (*B. globosus*), having parasite as *Schistosoma haematobium*, and causes human urinary schistosomiasis [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 [12]. The therapeutic applications of the plant have already documented in literature. It generally has antibacterial and antidiabetic and antioxidant properties [13]. The root and bark extracts were effective as laxative, and also used to relief stomach ache and piles [14, 15]. Haefliger et al. [16] reported that the plant was effective against fever and some urogenital tract diseases.

The plant was reported to have anti-venominal properties, also acute and oral administration of the plant to laboratory rats and mice suggested that the plant was safe [17]. Plants naturally have certain bioactive ingredients or metabolites which have found a broad range of valuable therapeutic application. For instance, some have been widely reported to possess repellent properties against mosquito [18], vectors of lymphatic filariasis [19] schistosomiasis [20], and several other pathogenic parasites [21].

Notwithstanding, there are several challenges encountered in the fight against vector-borne disease like malaria. They include but not limited to the ecotoxicity of synthetic pesticides [2, 20], re-infection after drug administration [22], as well as the rapid prolificacy of the vectors [23]. The application of synthetic pesticides against vectors that transmit diseases is not far-fetched. On the other hand, the problem associated with synthetic pesticides is the adverse impact against non-targeted species. As such, contemporary researches now focus on application of bioactive agents aimed at targeting vectors prior to maturity. The rationale of this research is to assay the seeds of *G. arborea* against vectors schistosomiasis and malaria, as a potential eco-friendly and bioavailable pesticidal strategy.

2. MATERIALS AND METHODS

2.1 Collection and preparation of plant materials

Fresh seeds of *G. arborea* were collected in Obio Akpor Local Government Area of Rivers State, Nigeria. The seeds were washed with de-chlorinated water and transported to the laboratory. Afterward the seeds were preserved in a foil plate at ambient temperature.

2.2 Express Extraction Process

Three hundred grams (300 g) of fresh seeds of the plant was weighed using Satoric AG Gottingen Electronic weighing balance. The weighed fresh seeds were pounded with clean ceramic mortar and pestle. Afterwards, the pounded seed was filtered into a clean and sterile conical flask to extract the juice, using whatman no.1 filter paper [24]. Prior to the bioassay, the obtained filtrate (i.e. juice) were preserved at room temperature.

2.3 Vector Collection/Breeding of Pulmonate and 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 (4.94886N 6.34046E; 4.93816N 6.34006E), using plastic container half-filled with water, and sand.

The baits were constantly monitored for the conspicuous emergence of larvae. Prior to the bioassay, the larvae were placed on an enamel tray with dechlorinated water (pH 7.4), and acclimatized to laboratory condition, using methods as described by Tiwari [25] and Dibua et al. [26].

The schistosomiasis vector snails of the genus *Bulinus* (*B. globosus*) and *Biomphalaria* (*B. pfeifferi*), were used for this bioassay. Pulmonates belonging to the *Biomphalaria* genus were collected from drainage along the Ahoda East-West road. The snails were transported to the laboratory, fed with water lettuce and bred in a plastic tank aquarium adjusted with aerator to improve oxygen supply. The applied method in this study which demonstrates the molluscicidal activity of the plant extract against the snails was developed using standard procedures [27].

2.4 Experimental Set Up

For the bioassay, samples of 20 larvae and snails, were distinctly placed in a 500ml solution of the extracts, in a 24-hour static test. It was performed in accordance with the World Health Organization guidelines [27]. 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 [26]. These screening protocols were carried out as described by Agboola et al. [22].

2.5 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 and/or snails at 10ppm during the rapid screening. The screening was carried out at different concentrations, in order to determine the minimal total lethal concentrations (LC₁₀₀).

2.6 Statistical Analysis

The data for mortality rates were expressed as mean± standard deviation using version 20 of SPSS statistical package. 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 (LC₅₀) of the seed sap against the larva and pulmonates (i.e snail), were estimated from the average minimal lethal concentrations in a concentration-mortality using Microsoft 2013 excel package.

3. RESULTS AND DISCUSSION

The mortality rates of all vectors (*An. gambiae*, *B. globosus* and *B. pfeifferi*), assayed against express seed sap extract of *G. arborea* are presented in Tables 1, 2 and 3. For the biolarvicidal bioassay, the positive control induced total mortality at 0.50ppm; on the other hand, the negative control demonstrated no mortalities against all assayed vectors (Table 1). Notwithstanding, varying degrees of mortalities were demonstrated between concentrations of 0.5 to 4.00 ppm (p<0.05). Statistically there was no significant difference (p>0.05) at 4.50 ppm (97.00%) and 5.00ppm (100%).

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

Conc. (ppm)	Mortality Rates (%) Mean \pm SD			95% Confidence Interval for Mean		Minimum	Maximum
	<i>A. gambiae</i>	Positive Control	Negative control	Lower Bound	Upper Bound		
0.00	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.0000	0.0000	0.00	0.00
0.50	26.36 \pm 1.72b	100.00 \pm 0.00h	0.00 \pm 0.00a	22.0873	30.6327	24.64	28.08
1.00	35.34 \pm 2.23c	100.00 \pm 0.00h	0.00 \pm 0.00a	29.8004	40.8796	33.11	37.57
1.50	39.32 \pm 1.87c	100.00 \pm 0.00h	0.00 \pm 0.00a	34.6747	43.9653	37.45	41.19
2.00	44.86 \pm 3.76d	100.00 \pm 0.00h	0.00 \pm 0.00a	35.5195	54.2005	41.11	48.63
2.50	55.72 \pm 2.64e	100.00 \pm 0.00h	0.00 \pm 0.00a	49.1619	62.2781	53.08	58.36
3.00	58.51 \pm 2.39e	100.00 \pm 0.00h	0.00 \pm 0.00a	52.5661	64.4672	55.98	60.74
3.50	63.83 \pm 3.95f	100.00 \pm 0.00h	0.00 \pm 0.00a	54.0177	73.6423	59.88	67.78
4.00	78.00 \pm 5.00g	100.00 \pm 0.00h	0.00 \pm 0.00a	65.5793	90.4207	73.00	83.00
4.50	97.00 \pm 2.00h	100.00 \pm 0.00h	0.00 \pm 0.00a	92.0317	101.9683	95.00	99.00
5.00	100.00 \pm 0.00h	100.00h \pm 0.00h	0.00 \pm 0.00a	100.0000	100.0000	100.00	100.00

For the biomolluscicidal bioassay, the positive control similarly induced total mortality at a concentration of 0.50ppm, while the negative control had no lethal effect against the vectors (Table 2). The bioassay indicated minimal total mortality at 4.00 ppm. Notwithstanding, mortality rates increased with a corresponding increase in concentrations between 0.50 – 3.50 ppm ($p < 0.05$). As

presented in Table 3, the biomolluscicidal assay against *B. pfeifferi* indicated varying degrees of mortalities between concentrations of 2.00 – 14.00 ppm ($p < 0.05$), while total mortality was demonstrated at 16.00 – 20.00 ppm with no significant difference ($p > 0.05$). It was also worthy of note that the negative control induced no mortality ($p > 0.05$), while the positive control was lethal at 2.00 ppm.

Table 2: Mortality rates for *B. globosus* molluscicidal Bioassay

Conc. (ppm)	Mortality Rates (%) Mean \pm SD			95% Confidence Interval for Mean		Minimum	Maximum
	<i>B. globosus</i>	Positive Control	Negative Control	Lower Bound	Upper Bound		
0.00	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.0000	0.0000	0.00	0.00
0.50	43.36 \pm 1.63b	100.00 \pm 0.00g	0.00 \pm 0.00a	39.3109	47.4091	41.73	44.99
1.00	54.34 \pm 2.30c	100.00 \pm 0.00g	0.00 \pm 0.00a	48.6265	60.0535	52.04	56.64
1.50	59.32 \pm 4.99d	100.00 \pm 0.00g	0.00 \pm 0.00a	46.9242	71.7158	54.33	64.31
2.00	62.84 \pm 1.79d	100.00 \pm 0.00g	0.00 \pm 0.00a	58.3934	67.2866	61.05	64.63
2.50	71.12 \pm 2.18e	100.00 \pm 0.00g	0.00 \pm 0.00a	65.7046	76.5354	68.94	73.30
3.00	74.49 \pm 3.11e	100.00 \pm 0.00g	0.00 \pm 0.00a	66.7683	82.2251	71.67	77.83
3.50	80.00 \pm 0.77f	100.00 \pm 0.00g	0.00 \pm 0.00a	78.0872	81.9128	79.23	80.77
4.00	100.00 \pm 0.00g	100.00 \pm 0.00g	0.00 \pm 0.00a	100.0000	100.0000	100.00	100.00
4.50	100.00 \pm 0.00g	100.00 \pm 0.00g	0.00 \pm 0.00a	100.0000	100.0000	100.00	100.00
5.00	100.00 \pm 0.00g	100.00 \pm 0.00g	0.00 \pm 0.00a	100.0000	100.0000	100.00	100.00

Table 3: Mortality rates for *B. pfeifferi* molluscicidal Bioassay

Conc. (ppm)	Mortality Rates (%) Mean±SD			95% Confidence Interval for Mean		Minimum	Maximum
	<i>B. pfeifferi</i>	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
2.00	33.36±1.85b	100.00±0.00h	0.00±0.00a	28.7643	37.9557	31.51	35.21
4.00	41.34±2.95c	100.00±0.00h	0.00±0.00a	34.0118	48.6682	38.39	44.29
6.00	45.32±1.45d	100.00±0.00h	0.00±0.00a	41.7180	48.9220	43.87	46.77
8.00	47.84±1.23d	100.00±0.00h	0.00±0.00a	44.7845	50.8955	46.61	49.07
10.00	56.12±3.24e	100.00±0.00h	0.00±0.00a	48.0714	64.1686	52.88	59.36
12.00	73.87±3.78f	100.00±0.00h	0.00±0.00a	64.4800	83.2600	70.09	77.65
14.00	80.00±1.92g	100.00±0.00h	0.00±0.00a	75.2305	84.7695	78.08	81.92
16.00	100.00±0.00h	100.00±0.00h	0.00±0.00a	100.0000	100.0000	100.00	100.00
18.00	100.00±0.00h	100.00±0.00h	0.00±0.00a	100.0000	100.0000	100.00	100.00
20.00	100.00±0.00h	100.00±0.00h	0.00±0.00a	100.0000	100.0000	100.00	100.00

The biolarvicidal activities of all assayed vectors using the fresh seed sap express extracts of *G. aborea* against the vectors (*An. gambiae*, *B. globosus* and *B. pfeifferi*), is presented in Figures 1, 2 and 3. As presented in figure 1, the seed sap extract against the larva vector belonging to Anopheles genus (*An. gambiae*), had LC50 values of 2.25 ppm (Figure 1). The low LC50 value demonstrated by the sap against the larvae is an indication of its larvicidal potentials. Meanwhile, the in the negative control induce no mortality against the larvae, but the on the other hand positive control was lethal to larvae at all assayed concentrations.

Furthermore, the activities of the plant extracts against *B. globosus* and *Biomphalaria pfeifferi* had LC50 values of 0.75 ppm (Figure 2) and 8.00ppm (Figure 3) respectively. It was noteworthy that the *Biomphalaria* specie was less susceptible to the biolarvicidal agent, compared to the *Bullinus* species (*B. globosus*). This variation is largely attributed to disparities in their genetic makeup, since they belong to different genus. Meanwhile, the *B. globosus* is the vector for urinary schistosomiasis, while *B. pfeifferi* is responsible for intestinal schistosomiasis. Notwithstanding, the negative control induced no mortality, while the positive control was lethal at all assayed concentrations.

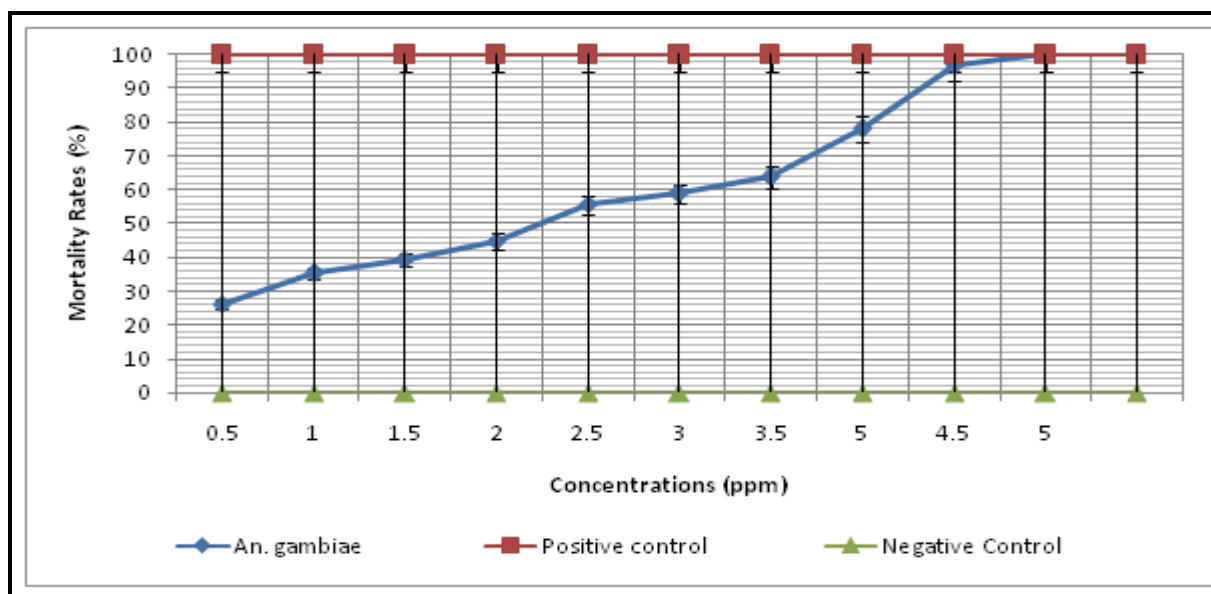


Figure 1: Concentration-mortality of express seed sap extracts of *G. arborea* against *An. gambiae*

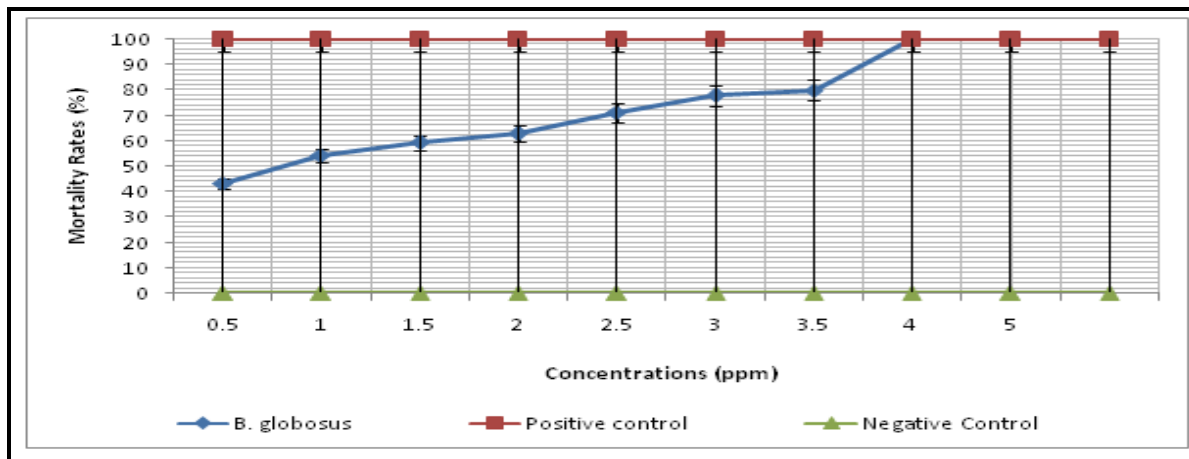


Figure 2: Concentration-mortality of express seed sap extracts of *G. arborea* against *B. globosus*

A recent study on the biolarvicidal activities of the leaf-crude extract of *G. arborea* against *An. gambiae* demonstrated mortality rates of 48.89 - 52.44% in 4 hours, 54.32 - 61.61% in 8 hours and 71.11 - 79.43% in 12 hours [28]. In the same study, other solvent extracts like methanol (45.43 - 56.36%, 58.17 - 71.86% and 88.59 - 99.94%), ethanol (48.89 - 56.44, 58.17 - 71.86 and 79.91 - 89.84%), hexane (44.99 - 52.49, 51.84 - 63.21 and 75.87 - 84.83%) and chloroform (44.99 - 52.49, 51.84 - 63.21, and 75.87 - 84.83%) were also reported.

There are several phytochemical in *G. arborea* already established in literature by several authors [28, 29].

These phytochemicals which were reported to support the bioactivities of the plant include; flavonoid, alkaloids, arboreal, isoarboreal, methyl arboreal, glummadiol, gmelanone, n-hexacosnol, sitostereol and hutteolin. The antimicrobial activitie of plant was also reported against some pathogenic microbes [29 - 31]. Also, phytochemical of *G. arborea* were reported to have anti-malaria, and analgesic properties [29]. All aforementioned literatures had acknowledged the fact that the bioactive metabolites in the plant had supported their activities against vectors and pathogens.

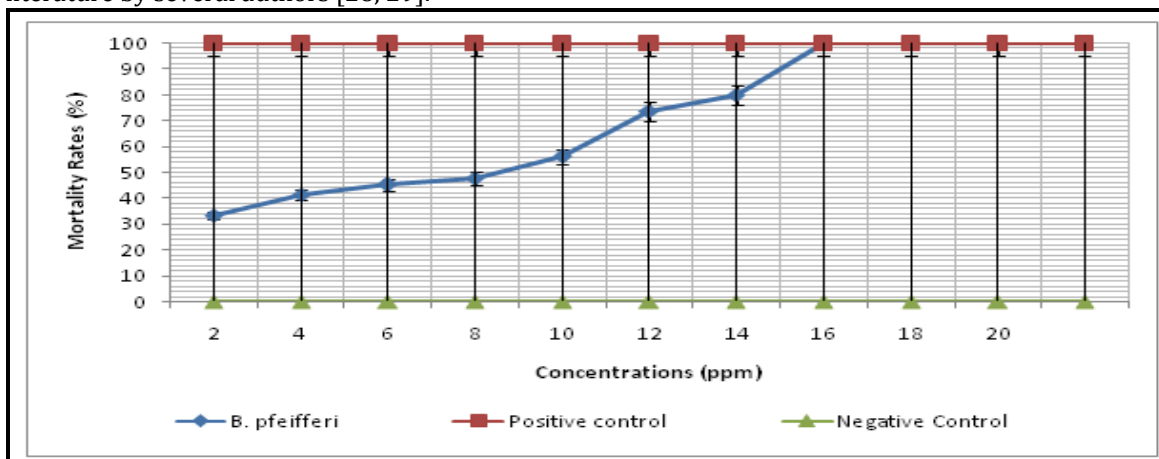


Figure 3: Concentration-mortality of express seed sap extracts of *G. arborea* against *B. pfeifferi*

4. CONCLUSIONS

Express seed extract of *G. arborea* was investigated for their biocidal activities against vectors of malaria and schistosomiasis. The rationale of this study was to derive an eco-friendly an alternative control of vector-borne diseases, which have become a global incident. Fortunately, from the results it can be concluded that the aforementioned plant was significantly lethal to the vectors in at a moderate concentration. Based on results of this finding and other corroborating literatures; it is also recommended that the cultivation of *G. arborea* be encouraged, in anticipation of their integrated trial to determine their actual field efficacy.

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