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Biolarvicidal Potentials of the Methanolic-Leaf-Extracts of Selected Tropical Plant Species

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Abstract

The global impact of malaria and challenges encountered during its control have necessitated the application of multifaceted strategies, including the application of plant-derived agents. Amidst these challenges the proliferation of the vector is becoming hyperendemic in tropical region. This research is focused on the biolarvicidal activities of the methanolic leaf-extracts of *Cassia alata*, *Microdesmis puberula*¸ and *Spilanthes filicaulis* against mosquito larva. The mosquito larva were assayed in a static non-renewal test. Results showed no mortality for the negative control, and total mortality for the positive control ($p<0.05$). The *C. alata* bioassay was the most active with LC_{50} value of 13.73 ppm, followed by; *M. puberula* (21.24 ppm), and *S. filicaulis* (28.86 ppm). This study concludes that methanolic-leaf-extracts of *C. alata*, *M. puberula*¸ and *S. filicaulis* can be recommended for the formulation of biolarvicide for the control of malaria.

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Introduction

Malaria is a vector-borne disease, which is transmitted by female arthropod (mosquito) belonging to the *Anopheles* genus [1, 2]. The disease is prevalent in Africa, with documented information in literature indicating, the *Anopheles gambiae* species as the predominant transmitter [3]. It is documented in literature that mosquitoes transmit more diseases, compared to other arthropods [4]. Statistical data in literature showed that there are over 40 genera of mosquitoes distributed into over 3,000 species, out of which only about 30 - 40 species transmit malaria in nature [2, 5].

Several metabolites have been identified from various plants, including over 10,000 alkaloids and 25,000 terpenes derivatives [6]. Notwithstanding, the therapeutic efficacies in these applied metabolites varies upon some compounding parameters; including, seasonal influence, location, age, individual susceptibility and environmental stresses on the plant [7], or the applied part of the plant such as root, stem, fruits, leaves, and seeds [8], as well as the applied solvent medium used for the plant extraction [9].

The efficacy of *C. alata* have been documented in literature for the treatment of constipation, stomach pain, and ringworm [10]. *Microdesmis puberula* was known for the treatment of gonorrhoea and erectile dysfunction [11]. The antibacterial properties of *Spilanthes filicaulis* leaf decoction have also been reported [12].

The application of synthetic therapy like drug administration can only abate morbidity burden and reinfection frequency [13], while pesticides poses potential ecotixicity [9]. Due to the prevalence of malaria and search for eco-friendly control measures, multifaceted strategies have become necessary, including researches and application of plant-derived methods [2]. Therefore, the investigation on the larvicidal potential of *Cassia alata* have become necessary.

Material and Method

Collection and Preparation of Plant Samples

Fresh leaves of *C. alata*, *M. puberula* and *S. filicaulis* were collected around the vegetation of Wilberforce Island in Southern Ijaw Local Government Area of Bayelsa State, Nigeria. All plants were identified and washed with de-chlorinated water in the laboratory. Afterward, they were shade-dried at room temperature for 7 days. The shade-dried plants were placed in oven at 50°C for 30 minutes [2]. Before the solvent-extraction, the shade-dried leaves were distinctively powdered with electric blender.

Extraction Process

Three hundred grams (300 g) of the powdered leaves of each plants were weighed using Satoric AG Gottingen Electronic weighing balance. The weighed powdered leaves were distinctively macerated in 500 ml of the respective solvents (Chloroform, Hexane and Methanol) for 72 hours and filtered into conical flask using whatman No.1 filter paper [14]. The filtrates distinctly extracted using a rotary evaporator at 60°C. The residue of the extracts was allowed to cool and stored at room temperature.

Mosquito Larva Collection

Mosquito Larvae used for this bioassay were cultured in the wild using methods as described by some authors [3, 8, 9, 12], with slight modification. Plastic containers and automobile tyres half-filled with stagnant water, and sand were kept in vegetation of conspicuous breeding sites. Prior to the laboratory bioassay, the larvae were placed on enamel tray and acclimatized to laboratory condition.

Experimental Setup

A minimum of 10 larvae, were distinctly placed in a 500 ml solution of the methanolic-extract at varying concentrations, in a 24-hour static non-renewal test. The bioassay was performed with the standard of the World Health Organization guidelines [12, 15]. Mortality rates (%), of larvae was recorded after the period of exposure (24 hours). A concentration of 1 ppm of Dipex pesticide was used as the positive control, while 500 ml of distilled water was used as the negative control. The larvicidal screening protocols was two-phased, involving the rapid and final Screening [12].

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.

Results and Discussion

The mortality rates of all methanolic leaf extracts of the plants (*C. alata*, *M. puberula* and *S. filicaulis*), assayed against the larvae is presented in Tables 1. Results showed that the positive control had total mortality at concentration below 10.00 ppm, while the negative control demonstrated no mortalities against the vectors (Table 1). For the *Cassia alata* bioassay results showed that mortality rate increased significantly with higher concentration ($p<0.05$). However, mortality rates ranging from 46.66 – 100.00%, with the minimal and total minimal mortality rates at concentrations 10 and 60 ppm respectively (Table 1).

The no adverse effect level was observed at concentration of 0 ppm. Furthermore, based on dose-response curve the *Cassia alata* bioassay had biolarvicidal activity with LC_{50} value of 13.72 ppm (Figure 1). The result for the methanolic-leaf-extract bioassay of *M. puberula* showed that mortality rate significantly ranged from 30.00 – 100.00% as presented in Table 1. The minimal mortality rate was observed at concentration of 10 ppm, while the total minimal mortality rate was observed at concentration of 70 ppm (Table 1). Meanwhile no adverse effect level was at concentration of 0 ppm (Table 1). Based on the dose-response the activity of methanolic leaf extract of *M. puberula* was demonstrated with LC_{50} value of 21.24 ppm (Figure 1). Results of the *S. filicaulis* methanolic-leaf-extract showed that mortality rates ranged from 16.66 – 100.00% ($p<0.05$). The minimal and total minimal concentration were 10 and 80 ppm respectively (Table 1), with LC_{50} value of 28.86 ppm (Figure 1).

Results of this study is comparable to a recent study for the larvicidal methanolic leaf (32.13 – 100.00%) and Hexane flower of *S. alata* (21.94 – 100.00%) of *Senna alata* (16). The

Table 1. Biolarvicidal Mortality rates of Methanolic-leaf-extracts

Conc.	Mortality Rates (%)				
	C. alata	*M. puberula*	*S. filicaulis*	Positive	Negative
0 ppm	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
10 ppm	46.66±11.54b	30.00±10.00b	16.66±5.77b	100.00±0.00e	0.00±0.00a
20 ppm	56.66±5.77bc	43.33±5.77c	33.33±5.77c	100.00±0.00e	0.00±0.00a
30 ppm	63.33±5.77cd	53.33±15.27c	43.33±11.54d	100.00±0.00e	0.00±0.00a
40 ppm	73.33±11.54d	66.66±5.77d	53.33±5.77e	100.00±0.00e	0.00±0.00a
50 pm	90.00±10.00e	80.00±10.00e	66.67±5.77f	100.00±0.00e	0.00±0.00a
60 ppm	100.00±0.00e	96.66±5.77f	76.66±5.77g	100.00±0.00e	0.00±0.00a
70 ppm	100.00±0.00e	100.00±0.00f	93.33±5.77h	100.00±0.00e	0.00±0.00a
80 ppm	100.00±0.00e	100.00±0.00f	100.00±0.00h	100.00±0.00e	0.00±0.00a
90 ppm	100.00±0.00e	100.00±0.00f	100.00±0.00h	100.00±0.00e	0.00±0.00a
100 ppm	100.00±0.00e	100.00±0.00f	100.00±0.00h	100.00±0.00e	0.00±0.00a

Data expressed as mean ± standard deviation, differences in alphabetical subscript indicates significance in mortality

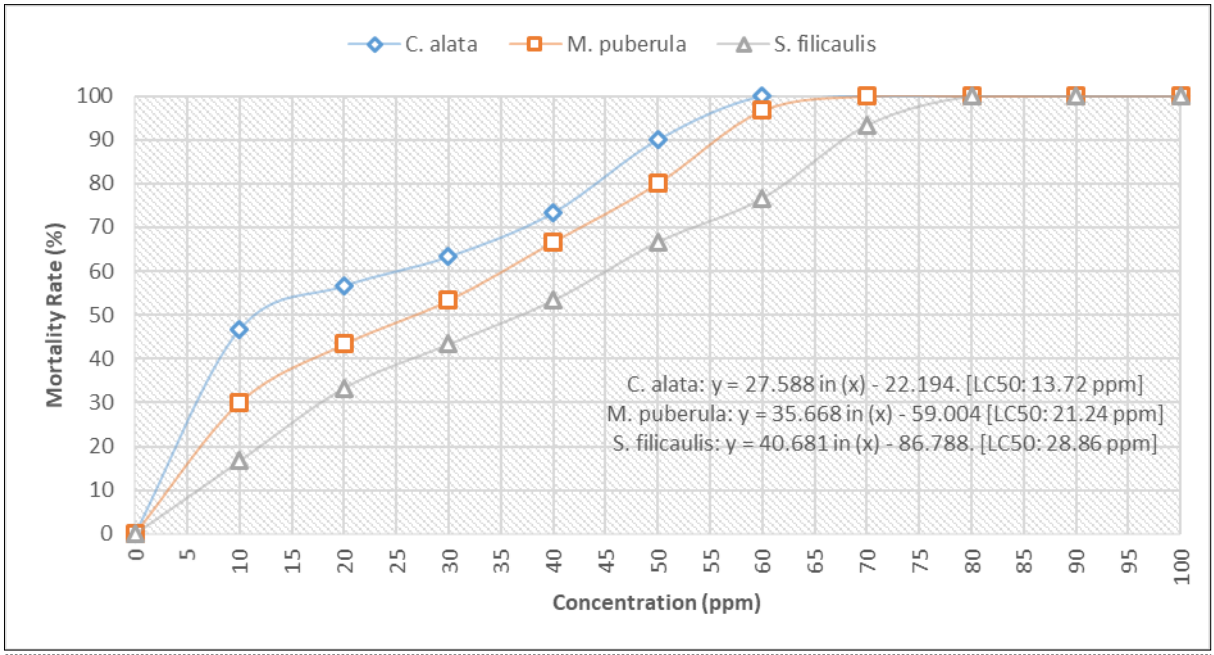

Figure 1. Biolarvicidal dose-response for methanolic-leaf-extracts of selected plant species

comparative larvicidal efficacies of the leaves, bark, stem and root of *Jatropha curcas* (Euphorbiaceae) against 3rd and 4th instar larvae *Anopheles gambiae* was reported by Ohimain *et al.*, [9]. Results of their studies demonstrated activities with LC_{100} and LC_{50} values for crude extract of the bark (437.5 and 87.5 ppm), methanolic-extract of the root (312.5 and 62.5 ppm), methanolic-extracts of the stem (237.5 and 47.5 ppm), leaves (75 and 15 ppm), and the bark (30 and 6 ppm), which was the most active.

The larvicidal activities of solvent-extracts of *Hyptis suavolens* and *Ocimun sanctum* were investigated against mosquito larva by Ohimain *et al.*, [17]. Results showed that the methanolic-extract of *H. suaveolens* induced was the most active with LC_{50} values of 73.25 ppm, followed by the chloroform and hexane extracts that demonstrated activities with LC_{50} values of 76.25 and 97.25 ppm respectively. Comparatively, the *O. sanctum* bioassay demonstrated activities LC_{50} values of 125.00 for methanolic-extract, 150.00 for chloroform-extract and 194.08 ppm for hexane-extract.

Several authors have reported phytochemicals in *C. alata*, *M. puberula*, and *S. filicaulis* that supported their antimicrobial activities [18, 19]. Phytochemical like alkaloid, flavonoid, saponin, tannin and phenol were predominantly found in the leaves of *C. alata* [20]. Several author have confirmed the phytochemistry of *M. puberula*, this include phytochemicals like; alkaloid, flavonoid, saponin and steroids [20]. The activities of *S. filicaulis* have been ascribed to predominant phytochemicals such as athraquinones, alkaloid, flavonoid, tannin and phenol [15, 19, 20].

Conclusion

This study investigated the biolarvicidal potential of the methanolic extracts of *C. alata*, *M. puberula*¸ and *S. filicaulis*. Intrestingly, all extracts of the plant showed larvicidal activities with the *C. alata* extract exhibiting higher mortality, followed by *S. filicaulis* and *M. puberula*. Solvent methanolic-extracts of these plants, are hereby recommended for the formulation of biolarvicide for the control of malaria.

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