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Biolarvicidal control of malaria using solvent extracts of *senna alata*

Abstract

The application of plant-derived pesticide have become a necessity due to the toxicity induced by synthetic pesticide and the proliferation of mosquito in endemic area. This research focused on the biolarvicidal assessment of methanol and hexane extracts of the leaf and flower of *Senna alata* plant. Mosquito larva were collected from the wild and assayed against for mortality efficacies using a static non-renewal test. Results showed that the negative control induced no mortality, while the positive control was lethal ($p<0.05$). The leaf Flower of the plant demonstrated higher mortality rate than the flower for both methanolic and hexane extracts ($p<0.05$). This study, concludes that both methanolic and hexane extracts of the leaf and flower of *S. alata* can be recommended for the formulation of biolarvicide for the control of malaria.

Keywords: mosquito, malaria, *senna alata,* larvcide, solvent extract

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Introduction

Malaria disease is transmitted by female mosquito belonging to the genus *Anopheles*.[1,2] However, in Africa, the predominant and most endemic vector for malaria transmission is *Anopheles gambiae*.[3] It is documented in literature that mosquitoes transmit more diseases, compared to other arthropods.[4] The National Centre for Infectious Diseases-NCID,[5] reported that over 3,500 species of mosquitoes belonging to over 40 genera exist in nature, out of about which 430 species belongs to the *Anopheles* genus, of which only about 30-40 species transmit malaria in nature.[2] The plant *Senna alata* otherwise Candle Bush or ringworm tree, is an important medicinal and ornamental flowering shrub plants belonging to the Caesalpiniodeae subfamily. The conventional application of synthetic drugs in the fight against malaria has been commendable. However due to the endemic nature of the disease, integrated comprehensive approach which includes control of the its vector, prior to maturity has become necessary.

Previous studies have shown that *S. alata* have antimicrobial properties against some pathogenic fungi,[6] and bacteria,[7] as well as antioxidant activities.[8] Besides the fact that on the other hand, synthetic therapy drug administration can only abate morbidity burden and reinfection frequency.[9] In addition, synthetic pesticide still pose some eco-toxic potential, as they could affect non-targeted species.[10] Due to the endemic trend of malaria the strategies adopted for it control has become diverse in nature. As reported in several literatures, researches on plant-derived drugs for their combat against major diseases of public health have become invoke.[2] As such it has become necessary to investigate the larvicidal potential of *Senna alata*.

Material and method

Collection and preparation of plant sample

Fresh leaves, flower and root of *S. alata* shall be collected around the bushes of Otuoke Community in Ogbia Local Government Area of Bayelsa State, Nigeria. All plant parts shall be gently washed with de-chlorinated water and transported to the laboratory, where they shall be shade-dried at room temperature for 72hours. Afterward the shade-dried plants were placed in oven at 50°C for 30minutes.[2] Prior to solvent extraction, the root shall be pounded with mortar and pestle, while the leaves and flower shall be distinctively powdered with an electric blender.

Extraction process

Three hundred grams (300g) of the powdered plants (i.e leaves, flower and root), shall be weighed using Satoric AG Gottingen Electronic weighing balance. The weighed plants shall be distinctively macerated in 500ml of the respective solvents (Methanol and Hexane) for 72hours. The concoctions shall be filtered into conical flask using whatman no.1 filter paper.[11] Filtrates of the macerated concoctions shall be respectively extracted using a rotary evaporator at 60°C. The obtained extracts shall be allowed to cool and preserved for the bioassay at low temperature (4°C).

Mosquito larva collection

Mosquito Larvae belonging to the genus *Anopheles*, shall be used for the purpose of this research. The larvae were cultured in the wild using methods as described by several authors;[10,12,13] Plastic containers and automobile tyres half-filled with stagnant water, and sand shall be positioned as the bait in bushes and conspicuous breeding sites. The baits shall be constantly monitored for emergence of larvae. Prior to the laboratory bioassay, the larvae shall be placed on enamel tray of dechlorinated water (pH 7.4), in order to acclimatized to laboratory condition.[12]

Experimental setup

For the larvicidal bioassay, a minimum of 20 larvae, shall be distinctly placed in a 500ml solution of the extracts at varying concentrations, in a 24-hour static non-renewal test. It was performed in accordance with the World Health Organization guidelines (WHO, 1996). The mortality rates (%), of larvae shall observed and recorded within a period of 24hours. One part per million (1 ppm) of Dipex pesticide shall be used as the positive control, while 500ml of distilled water adjusted with 2.5ml of 10% dimethyl sulfoxide (DMSO) at pH 7.5, was used as the negative control.[12] These screening protocols shall

be undertaken in two phases (Rapid and Final Screenin), described by Agboola et al.,[9] with slight modification.

Biolarvicidal screening

For the purpose of the bioassay all investigation shall be carried out in triplicates. For the rapid screening test, triplicate concentrations of varying concentration ranging from 500 - 1000ppm shall be used to screen the larva for total (i.e. 100%) mortality within 24hours 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 500ppm during the rapid screening, shall be further screened in order to determine the minimal lethal concentration.

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.

Result and discussion

The mortality rates of all methanolic leaf extracts of the plants (Leaves and Flower), 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 leaf extract results showed that mortality rate increased significantly with higher concentration ($p<0.05$). The no adverse effect level (NOAEL) was observed at concentration of 0 ppm. However, mortality rates ranging from 41.23 – 100.00%, with the minimal and total minimal mortality rates at concentrations 10 and 70ppm respectively (Table 1).

The result for the methanolic flower of *S. alata* showed that mortality rate significantly ranged from 26.14 – 100.00% as presented in Table 1. The minimal mortality rate was observed at concentration of 10 ppm, while the minimal total mortality rate was observed at concentration of 70 ppm (Table 1). Meanwhile no adverse effect level was at concentration of 0 ppm. The minimal and total minimal mortality rates were also observed at concentrations 10 and 70 ppm respectively, with lower mortality rates compared to the methanolic leaf extract (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. The mortality rates of all Hexane leaf extracts of the plants (Leaves and Flower), assayed against the larvae is presented in Tables 2. Results showed that the positive control had total mortality at concentration below 10.00ppm, while the negative control demonstrated no mortalities against the vectors (Table 1). For the leaf extract results showed that mortality rate increased significantly with higher concentration ($p<0.05$). The no adverse effect level (NOAEL) was observed at concentration of 0ppm. However, mortality rates ranging from 32.13 – 100.00%, with the minimal and total minimal mortality rates at concentrations 10 and 80 ppm respectively (Table 2).

Table 1 Biolarvicidal Mortality rates for Methanolic extracts

Concentrations	Leaf	Flower	Positive control	Negative control
0 ppm	0.00±0.00	0.00±0.00	0.00±0.00a	0.00±0.00
10 ppm	41.23±7.53	26.14±6.10	100.00±0.00	0.00±0.00
20 ppm	43.41±4.27	33.13±9.57	100.00±0.00	0.00±0.00
30 ppm	53.23±3.77	43.33±15.27	100.00±0.00	0.00±0.00
40 ppm	61.23±9.34	54.21±9.67	100.00±0.00	0.00±0.00
50 pm	73.19±14.17	68.34±17.31	100.00±0.00	0.00±0.00
60 ppm	91.70±17.20	79.27±11.07	100.00±0.00	0.00±0.00
70 ppm	100.00±0.00	100.00±0.00	100.00±0.00	0.00±0.00
80 ppm	100.00±0.00	100.00±0.00	100.00±0.00	0.00±0.00
90 ppm	100.00±0.00	100.00±0.00	100.00±0.00	0.00±0.00
100 ppm	100.00±0.00	100.00±0.00	100.00±0.00	0.00±0.00

Data expressed as mean mortality rate ± standard deviation

Table 2 Biolarvicidal Mortality rates for Hexane extracts

Concentrations	Leaf	Flower	Positive control	Negative control
0 ppm	0.00±0.00	0.00±0.00	0.00±0.00a	0.00±0.00
10 ppm	32.13±9.19	21.94±11.20	100.00±0.00	0.00±0.00
20 ppm	39.11±8.72	27.73±17.09	100.00±0.00	0.00±0.00
30 ppm	42.71±7.91	35.51±13.72	100.00±0.00	0.00±0.00
40 ppm	48.13±11.30	39.97±10.67d	100.00±0.00	0.00±0.00
50 pm	66.29±24.19	56.14±17.31	100.00±0.00	0.00±0.00
60 ppm	87.67±31.10	69.94±19.07	100.00±0.00	0.00±0.00
70 ppm	97.41±22.20	88.93±24.19	100.00±0.00	0.00±0.00
80 ppm	100.00±0.00	100.00±0.00	100.00±0.00	0.00±0.00
90 ppm	100.00±0.00	100.00±0.00	100.00±0.00	0.00±0.00
100 ppm	100.00±0.00	100.00±0.00	100.00±0.00	0.00±0.00

Data expressed as mean mortality rate ± standard deviation

The result for the Hexane flower of *S. alata* showed that mortality rate significantly ranged from 21.94– 100.00% as presented in Table 1. The minimal mortality rate was observed at concentration of 10ppm, while the minimal total mortality rate was observed at concentration of 70 ppm (Table 1). Meanwhile no adverse effect level was at concentration of 0ppm. The minimal and total minimal mortality rates were also observed at concentrations 10 and 70ppm respectively, with lower mortality rates compared to the methanolic leaf extract (Table 2). The plant contains diverse phytochemicals that supported its activity. Besides, the therapeutic efficacies of *S. alata* had been previously documented in literature for treatment of Pityriassis versicolor.[14] The antimicrobial activity of ethanol extract of the leaf of *S. alata* was also reported with zones of inhibition for; Kiebsiella spp (27.4mm), Salmonella typhi (26mm), Pseudomona auriginosa (26mm), Protus mirabilis (21.7mm), and E. coli with 19.5mm as reported by Zige et al.[15]

Conclusion

This study investigated the biolarvicidal potential of the leaf and flower, using methanol and hexane extracts. Fortunately, both extracts of the plant demonstrated larvicidal activities with the leaf extract having higher mortality rate compared to the flower. In addition, the methanolic extract was more effective that the hexane extract. Notwithstanding, the larvicidal efficacy of S. alata plant indicated that it can be recommended for the formulation of biolarvicide for the control of malaria.

Acknowledgments

None.

Conflicts of interest

The author(s) declares that there is no conflicts of interest.

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