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Repellent potentials of *Securidaca longepedunculata* Fresen (Polygalaceae) crude extract and essential oil against mosquitoes

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Abstract

This study aimed to evaluate the bioactivities of the plant, *Securidaca longepedunculata* Fresen crude extract and essential oil against two different species of mosquitoes; *Anopheles gambiae* s.l. and *Culex quinquefasciatus*. Ten (4 to 7 days old) non-blood fed female mosquitoes, from each of the species were exposed to test plant- treated chamber linked to an inner untreated chamber for 10 minutes. Parameter assessed was behavioural response of test insects to treatment (test plants). There was a significant difference ($P < 0.05$) between the behavioural response of both test insects to essential oil. Both crude extract and essential oil at 0.3 and 0.5 mg/ml concentration recorded higher percentage repellency against *Anopheles gambiae* s.l. than the standard treatment, DEET (N, N-diethyl-m-toluamide), while only 0.5mg/ml concentration of crude extract, recorded percentage repellency higher than what was recorded in DEET against *Culex quinquefasciatus*. These findings show the repellent potentials inherent in the test plant, thus qualifying it as a suitable alternative to synthetic chemical repellents. However further investigation is suggested to examine the mode of action of extracted substances which could facilitate the formulation of a better herbal repellent.

Keywords: Mosquitoes, *Securidaca longepedunculata*, crude extract, essential oil, repellency

1. Introduction

Mosquitoes are found throughout the world, particularly in tropical and sub-tropical climates [1]. In Nigeria, mosquitoes are abundant in marshy lands, near collected waters, ponds, stagnant water bodies etc., and the medically important species includes *Anopheles* spp. which is a vector of *Plasmodium* sp. which causes malaria and the *Culex* spp. incriminated with the transmission of filarial worms, that causes filariasis [2]. Malaria is responsible for the high infant mortality rates among populations in the tropical and sub-tropical climates of the world [2]. The basic epidemiology of malaria involves man to *Anopheles* to man transmission cycle, in which man acts as a sole intermediate host and reservoir of the parasite, *Plasmodium* sp. and mosquito as the definitive host. Filariasis on the other hand is also a deadly tropical disease that has negatively impacted on many lives in Nigeria [2]. The application of synthetic chemicals which appears to be a major control measure presently raises both human health and environmental deterioration concerns. The introduction of synthetic repellents such as N, N-diethyl-m-toluamide (DEET) which provided relatively longer protection time against haematophagous insects, however led to complete abandonment of many indigenous repellent plants which formed the basis of most commercially produced repellents existing before the advent of DEET [3, 4]. Globally and recently, there has been growing interest in research concerning the use of plants extracts as alternatives to synthetic insecticides in pest management [5-7]. Plant derived materials are more readily biodegradable, less toxic to mammals, retard the development of resistance, easy and cheap to produce by farmers and small-scale industries as crude or partially purified extracts [8-10]. Various studies show that these two important insect vectors, *Anopheles gambiae* s.l. and *Culex quinquefasciatus* are strongly repelled by some synthetic toxic materials to the extent that they become disoriented [11-13]. The present work seeks to evaluate the repellent potency of a local non-toxic medicinal plant, *Securidaca longepedunculata* Fresen against *Anopheles gambiae* s.l. and *Culex quinquefasciatus* as a reliable alternative to chemical repellents.

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2. Materials and Methods

2.1 Collection of plants/ Extraction

Fresh roots of *Securidaca longepedunculata* Fresen (Polygalaceae) were obtained from a field around the University of Ilorin water dam, situated between Latitude 8° 28' 5" N and Longitude 4° 40' 0" E. The plant material was selected on the basis of its ethno-medical and endemicity. It was collected in a treated cellophane bag and taken to the herbarium unit of the Department of Plant Biology, University of Ilorin where it was identified and authenticated. The plant was thereafter air-dried and pulverized. The pulverized plant materials were then extracted separately with 20g/ml methanol by maceration for 48 to 72 hours. The extracts were filtered using cotton wool and Whatman filter paper separately to remove coarse particles [12]. Essential oil was decanted while extracts were air-dried at room temperature to remove all traces of solvents.

2.2 Mosquito Rearing

A susceptible colony of *An. gambiae* s.l. and *Cu. quinquefasciatus* mosquito strains were established at the Entomology laboratory, Department of Zoology, University of Ilorin. The mosquitoes were reared following the [14] standard procedures for insect breeding. The mosquito colony was maintained in a climatic controlled room at 27 ± 2 °C, 80 ± 10 RH and with a photoperiod cycle of 12h light: 12h dark. Mosquito larvae were fed a diet of no-fatty biscuit mixed with yeast while emerged adults were aspirated mechanically into holding cages and provided with 10% sugar solution.

2.3 Preparation of stock solution and test concentrations

The volume of stock solution was prepared by weighing out 100 mg of the extracts and 1 ml of the essential oil and dissolving in 10 ml of the solvent (acetone). The stock solution was then serially diluted (ten-fold) in the solvent (e.g. 1 ml stock solution to 9 ml solvent to form 0.1 g/ml etc.) to form test concentrations of different ranges (0.05mg/ml, 0.2mg/ml, 0.3mg/ml and 0.5mg/ml).

2.4 Repellency Bioassay

The apparatus used for this assay consisted of two connected tubes in the WHO test kit divided into two chambers, one treated and one untreated [15]. Different concentrations of the essential oil and extract from *Se. longepedunculata* roots (test product) were applied on Whatman filter papers and put into the treatment chamber while the inner surface of the untreated chamber was left bare. Ten non-blood-fed female *An. gambiae* s.l. and *Cu. quinquefasciatus* mosquitoes aged 4 to 7 days old were introduced into the treated chamber and after 30 seconds of the acclimation period, the sliding door separating the two chambers was opened for 10 minutes. At the end of the test period (10 minutes), the chamber was closed and the number of female mosquitoes in each chamber was recorded. This test was replicated three times for each test

product and concentration.

2.5 Data Analysis

The proportion of mosquitoes in treated and control were analysed using Students t-test, one-way Analysis of Variance (ANOVA) and Tukey's post-hoc test with the aid of Graph Pad Prism 8. The preference Index (PI) [15] was calculated using the formula below;

$$\text{Preference Index} = \frac{\text{Number of mosquitoes in Treated} - \text{Number of mosquitoes in Control}}{\text{Number of mosquitoes in Treated} + \text{Number of mosquitoes in Control}}$$

The percentage repellency [15] was calculated using the formula below;

$$\text{Percentage Repellency} = \frac{\text{Number in Treated} - \text{Number in Control}}{\text{Total Number}} \times 100$$

3. Results

The behavioural response of *Se. longepedunculata* essential oil on *Anopheles* and *Culex* mosquitoes (Table 1) revealed that 0.3 and 0.5mg/ml concentrations elicited the lowest mean number (0.00±0.00 mosquitoes) of *An. gambiae* s.l. mosquitoes in the treated chamber which showed better performance than that observed in the standard treatment DEET (0.67±0.58 mosquitoes). Meanwhile, 0.2mg/ml of the essential oil elicited 0.67±1.15 mosquitoes which showed similar effect as DEET on *An. gambiae* s.l. while 0.05mg/ml caused the highest mean number (1.00±1.00 mosquitoes) of *An. gambiae* s.l. in the treated chamber which was significantly lower than ($P < 0.05$) that of DEET. Similarly, at the same concentrations of 0.3 and 0.5mg/ml essential oil of test plant elicited the highest Preference Index, with PI of (-1.00) against *An. gambiae* s.l. which was higher than DEET (-0.87).

Essential oils of *Se. longepedunculata* at 0.2mg/ml elicited the lowest mean number of *Cu. quinquefasciatus* in the treated chamber (1.33±8.67 mosquitoes) followed by 0.5mg/ml (1.67±0.58 mosquitoes), 0.05mg/ml (2.00±1.00 mosquitoes) and the highest mean number was recorded in 0.3mg/ml (2.67±0.58 mosquitoes). However, DEET (0.67±0.58 mosquitoes) elicited better effect ($P < 0.05$) on *Cu. quinquefasciatus* when compared to all the concentrations of essential oil of *Se. longepedunculata* evaluated. Similarly, concentrations at 0.2mg/ml and 0.3mg/ml recorded the highest PI (-0.73) and lowest PI (-0.47) respectively against *Cu. quinquefasciatus*. All the PI's recorded were not comparable with the value obtained in DEET (-0.93). Interestingly, all the concentrations of the oil elicited significantly same response ($P < 0.05$) in both species of mosquitoes except 0.3mg/ml where there was a significant difference ($P > 0.05$) in what was recorded from the behavioural response of both species of mosquitoes.

Table 1: Behavioural response of *An. gambiae* s.l. and *Cu. quinquefasciatus* to *Se. longepedunculata* essential oil.

Conc. (mg/ml)	<i>Anopheles gambiae</i> s.l.			<i>Culex quinquefasciatus</i>			P value
	Untreated	Treated	PI	Untreated	Treated	PI	
0.05	9.00±1.00	1.00±1.00	-0.80	8.00±1.00	2.00±1.00	-0.60	0.288
0.2	9.33±1.15	0.67±1.15	-0.87	8.67±0.58	1.33±8.67	-0.73	0.422
0.3	10.00±0.00	0.00±0.00	-1.00	7.33±0.58	2.67±0.58	-0.47	0.001*
0.5	10.00±0.00	0.00±0.00	-1.00	8.33±0.58	1.67±0.58	-0.67	0.007
DEET	9.33±0.58	0.67±0.58	-0.87	9.67±0.58	0.33±0.58	-0.98	0.519

The behavioural response of *Se. longepedunculata* crude extracts on *Anopheles* and *Culex* mosquitoes (Table 2) revealed that at 0.3 and 0.5mg/ml lowest mean number of *An. gambiae* s.l. mosquitoes was recorded in the treated chamber (0.00±0.00 mosquitoes) which was better than ($P<0.05$) what was observed for DEET (0.67±0.58 mosquitoes). However, concentrations at 0.05 and 0.2mg/ml elicited the highest mean number of *An. gambiae* s.l. mosquitoes in the treated chamber (1.00±1.00 mosquitoes) which was significantly lower ($P<0.05$) to what was obtained in DEET. Crude extracts at 0.3 and 0.5mg/ml elicited the highest PI (-1.00) on *An. gambiae* s.l. mosquitoes which was higher than the value observed in DEET (-0.87). The lowest PI (-0.80) was also recorded in both 0.2 and 0.05mg/ml concentrations of crude extracts of the oil which is lower than what was observed in DEET. Crude extracts from *Securidaca longepedunculata* at 0.5mg/ml recorded the lowest mean number of *Cu.*

quinquefasciatus in the treated chamber (0.00±0.00 mosquitoes) which was better than ($P<0.05$) that of DEET (0.33±0.58 mosquitoes). However, crude extracts from *Se. longepedunculata* at 0.05 and 0.2mg/ml (0.33±0.58 mosquitoes) elicited same response as DEET on *Cu. quinquefasciatus*. However, crude extracts from *Se. longepedunculata* at 0.3mg/ml (0.67±0.58 mosquitoes) recorded the highest mean number of *Cu. quinquefasciatus* in the treated chamber. Similarly, the highest PI was recorded in 0.5mg/ml which was higher than what was obtainable in DEET (-0.93) while 0.05 and 0.2mg/ml recorded same PI as DEET against *Cu. quinquefasciatus*. The lowest PI was recorded in 0.3mg/ml (Table 2). Interestingly, all the concentrations of crude extracts from *Se. longepedunculata* elicited significantly same behavioural response ($P<0.05$) to both *Anopheles gambiae* s.l. and *Cu. quinquefasciatus*.

Table 2: Behavioural response of *An. gambiae* s.l. and *Cu. quinquefasciatus* to *Se. longepedunculata* crude extracts.

Conc. (mg/ml)	<i>Anopheles gambiae</i> s.l.			<i>Culex quinquefasciatus</i>			P value
	Untreated	Treated	PI	Untreated	Treated	PI	
0.05	9.00±1.00	1.00±1.00	-0.80	9.67±0.58	0.33±0.58	-0.93	0.374
0.2	9.00±1.00	1.00±1.00	-0.80	9.67±0.58	0.33±0.58	-0.93	0.374
0.3	10.00±0.00	0.00±0.00	-1.00	9.33±0.58	0.67±0.58	-0.87	0.116
0.5	10.00±0.00	0.00±0.00	-1.00	10.00±0.00	0.00±0.00	-1.00	NA
DEET	9.33±0.58	0.67±0.58	-0.87	9.67±0.58	0.33±0.58	-0.93	0.519

NA – Not Applicable

The results (Figures 1 A & B) also showed that both extracts and essential oil from the test plant at 0.3 and 0.5mg/ml concentrations elicited hundred percent repellency against *An. gambiae* s.l. and this was higher than what was observed in DEET (87.00%). Similarly, 0.5mg/ml of crude extract also elicited a hundred percent repellency against *Cu.*

quinquefasciatus which was higher than what was recorded in DEET (93.00%). Generally, *Se. longepedunculata* essential oil at 0.05, 0.2, 0.3 and 0.5mg/ml concentrations recorded very low percentage repellency of 60, 73, 47 and 67% respectively against *Cu. quinquefasciatus* which was lower to what was recorded in DEET (93%).

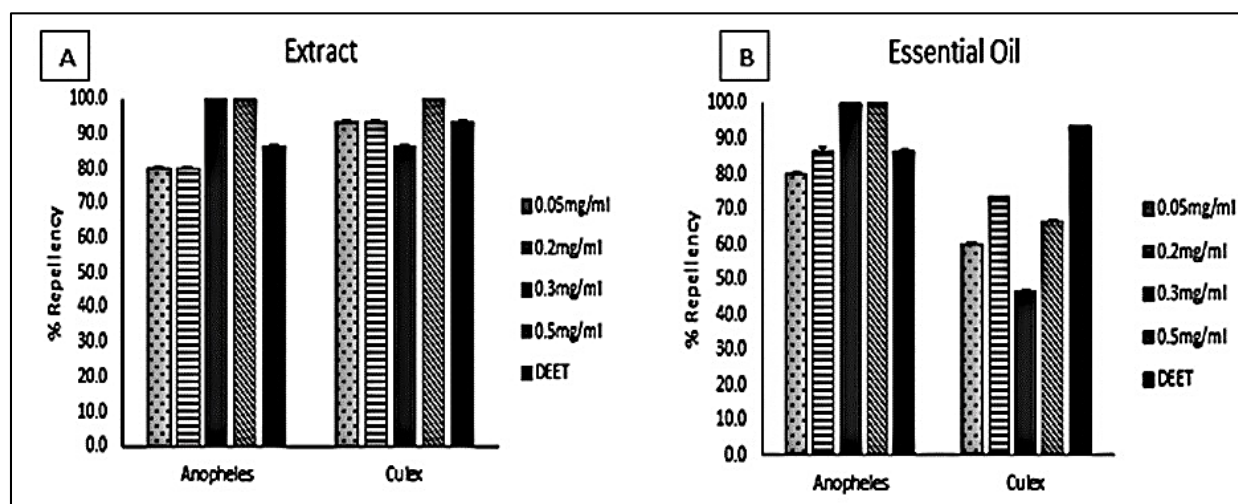


Fig 1: Percentage repellency of different concentrations of (A) crude extract and (B) essential oil of *Se. longepedunculata* against *An. gambiae* s.l. and *Cu. quinquefasciatus*

4. Discussion

This study dealt with the use, for the first time of the repellent activity of *Se. longepedunculata* root bark extracts against *Anopheles* and *Culex* mosquitoes. The findings clearly demonstrated the potency of the test plant crude extract and oil to cause significant ($p<0.05$) repellency of *An. gambiae* s.l. and *Cu. quinquefasciatus*. It is evident from our study that all bioassays and plant products from *Se. longepedunculata* tested reduced mosquito activity by making them to orientate

away from source hence, the plant displayed a form of repellent activity against both *Anopheles* and *Culex* mosquitoes. According to Erler *et al.* [13] and Taponjdjou *et al.* [16] using botanical materials as alternatives to chemical insecticides is an ideal method for insect pests' control as these plant derivatives have repellent, insecticidal, antifeedant and / or insect growth regulator effects. Essential oils and extracts of plant origin are emerging as potential agents for mosquito control because of their cost effectiveness,

availability, and biodegradable nature and environmental friendliness [11]. The result of this study is consistent with observations of Eziah *et al.* [17] who reported that extracts of the roots of *Se. longepedunculata* exhibited mean repellence of 60% and 80% against *Prostephanus truncatus* and *Tribolium castaneum* respectively at concentrations of 1.00 and 2.00g/ml. Similarly, Afful *et al.* [18] in their study observed that the extracts of the roots of *Se. longepedunculata* recorded mean percentage repellency of 70.1 and 60.3 of 0.10g/ml against *Callosobruchus maculatus* and *Sitophilus zeamais* respectively. In this study, 0.3 and 0.5mg/ml of *Se. longepedunculata* demonstrated better repellent activity against *An. gambiae* s.l. compared to that elicited by DEET, the positive control, while 0.2 and 0.05 mg/ml of same test plant demonstrated same repellent activity as DEET against *An. gambiae* s.l. All tested concentrations of essential oils from test plant did not elicit the measure of repellency demonstrated by DEET against *Cu. quinquefasciatus*, but the crude extract did. It was equally observed that 0.5mg/ml crude extract performed better than DEET against *Cu. quinquefasciatus*. These results corroborate the assertion of Mongalo *et al.* [10] that in their toxicity studies, both *in vivo* and *in vitro* extracts were only toxic at relatively higher concentrations. It is evident from this study that percentage mosquito repellency increased with higher dosages of oil which indicates direct relationship between the dose and percentage repellency. Previous studies showed that plant extracts brought into colonies of mosquitoes tended to repel them [3,4]. Singh *et al.* [19] reported that hexane extract of *Cyperus rotundus* was also effective in repelling three dipteran disease vectors, *An. quinquefasciatus*, *An. stephensi* and *Cu. quinquefasciatus*. The percent repellency at different observation periods (0, 1, 2, 4 and 6 hrs) ranged from 80 to 100% for different concentrations against different species. It was similarly reported by Kweka, *et al.* [20] that burning dry leaves of *Eucalyptus globulus* and *Lantana camara* showed deterrence rates of 88.1 and 79.4% respectively on *An. arabiensis* and 86.1 and 71.2% respectively against *Cu. quinquefasciatus*. It was also reported that essential oil *L. camara* flowers showed a strong repellent activity against *Aedes aegypti* [21]. The result of the present study confirms the aforementioned reports. A good number of plants possess self-defensive constituents that can exert action by combination of mechanism that includes iron chelation and enzymes inhibition; such action could repel, retard and even cause death of herbivores including vertebrates that feed on these plant substances.

The efficacy *Se. longepedunculata* to repel mosquitoes can be traced to the unique features they possess. They have a good history of usage as insecticides, medicines, antifungal and antiviral [22, 23]. Furthermore, the test plant potency to repel mosquitoes could also be attributed to the solvent used for its extraction; in this study, methanol was used as a solvent in the extraction of the plant substance. The success recorded in using methanol confirms the report of National Research Institute [24] that organic solvents such as methanol, ethanol, acetone etc. are five times more effective in extracting active ingredients from plants parts than aqueous extract. In the same vein, Adedire and Ajayi [25] and Arannilewa *et al.* [26] reported that extracts offer an additional advantage of being relatively efficacious against virtually all life stages of insects. Previous studies show that the physiological mechanism surrounding exposure of insects to essential oil and the

resultant effect in repelling or causing death is still a subject of disagreement among researchers. Whereas Singh *et al.* [19] reported that oil had no effect on repellency or death of adult bruchids, Obeng-Oferi [27] on the contrary suggested that repellency or death caused by essential oil is due to interference in normal respiration resulting in suffocation. However, according to Obeng-Oferi [27], oils could act as repellents, anti-feedants and subsequently discourage feeding and penetration.

5. Conclusions

This botanical, *Se. longepedunculata* should immediately be advocated for given the excellent repellent activity demonstrated against *An. gambiae* s.l. and *Cu. quinquefasciatus* mosquitoes. These plant substances are without any known health risks, are very easily available to people in rural areas and are widely used for its ethno-medical importance. Further investigation is however suggested to examine its effects on other species of mosquitoes and pests.

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7. Authors Contribution

EIO and OJA conceived the study and designed the laboratory work, drafted and review the manuscript for final approval. OJA and OAS executed the research work. OJA contributed to the data analysis and interpretation.

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