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Adulticidal and repellent activities of *Rhinacanthus nasutus* leaf extracts against *Aedes aegypti* Linn and *Culex quinquefasciatus* Say

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Abstract
To determine the adulticidal and repellent activities of different solvent leaf extracts of *Rhinacanthus nasutus* against *Aedes aegypti* and *Culex quinquefasciatus*. The Adulticidal efficacy of the leaf extracts of *Rhinacanthus nasutus* with four different solvents they are petroleum ether, ethyl acetate, chloroform and methanol and were tested against the five to six days old adult female mosquitoes of *Aedes aegypti* and *Culex quinquefasciatus*. The adult mortality was observed after 24h. The repellent efficacy was determined against *Aedes aegypti*, *Culex quinquefasciatus* at three different concentrations viz., 1.0, 2.5 and 5.0 mg/cm^2. Among the tested solvents the maximum efficacy was observed in the methanol extract. The LC_{50} and LC_{90} values of *Rhinacanthus nasutus* against adults of *Aedes aegypti* and *Culex quinquefasciatus* were 69.27, 73.47 ppm and 138.49 and 145.57 ppm respectively. Methanol extract of *Rhinacanthus nasutus* leaf extract produced maximum repellency in all the concentrations against two mosquito species at 240 min. The result concludes that *Rhinacanthus nasutus* serves as a potential agent for controlling *Aedes aegypti* and *Culex quinquefasciatus*.

Keywords: *Rhinacanthus nasutus*, Adulticidal, Repellent, *Aedes aegypti*, *Culex quinquefasciatus.*

1. Introduction
Mosquitoes cause more human suffering than any other organism because of their ability to vector pathogens of several diseases such as dengue fever, yellow fever, malaria, filariasis, Japanese encephalitis and other fevers [1]. Insecticides are the primary management tool for mosquitoes. However, high levels of pesticide resistance in mosquitoes can develop very quickly. There are over 125 mosquito species with documented resistance to one or more insecticides. Mosquito resistance to some insecticides has been documented within a few years after the insecticides were introduced [2]. In addition, there are heightened public concerns over synthetic pesticide use, such as fears about their effects on public health and negative environmental consequences. Several insecticides have been withdrawn for economic or regulatory reasons, and the cause of resistance. To overcome the problem of development of resistance in insects, attention is being given to natural products because of their biodegradable nature. A botanical phytochemical, with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, ovicidal, adulticidal and repellent properties [3].

Insect repellents are widely used as personal protection against biting arthropods. Personal protection measures, including the use of repellents are important in reducing the risk of contracting disease. The vector borne diseases cause a high level of morbidity and mortality, but they are also responsible for great socio-economical loss. The majority of commercial insect repellent preparations contain the chemical *N, N-diethyl-m-toluamide*, first synthesized in 1954 [4]. It has been reported that these chemical repellents are not safe for the public use. There has been much research on natural plant extracts both prior to and after the advent of synthetic repellents. Novak emphasized the urgent need of the investigation of phytochemical as repellents for mosquitoes in his review of non-chemical approach to mosquito control [5].

The *Rhinacanthus nasutus* commonly known as "Nagamalli" belongs to family *Acanthaceae*. The genus *Rhinacanthus* is comprised of about 25 species confined to the old world tropics and subtropics it is placed in the *Justiciinae* subtype [6]. *R. nasutus* is widely distributed in some parts of sub-continent, in the region of Southeast Asia and China [7]. The plant is a small slender shrub. The *R. nasutus* is cultivated particularly as a medicinal plant has been used in

treatments and preventions of diverse diseases as folklore medicines. Different parts of the plant have been used in traditional medicine for the treatment in diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension and several skin diseases [8]. The experimental evidences shows, that it has potential effects for treatment of cancer, liver disorders, skin diseases, peptic ulcers, helminthiasis, scurvy, inflammation and obesity [9]. The leaves of this plant are also used in the preparation of shampoos. *Rhinacanthine* from roots induce apoptosis in human cervical carcinoma cells and hepatocellular cancers [10-12]. Therefore, considering the significant importance of *R. nasutus* in folk medicine experiments were conducted on analysis of plant products and their biological activity.

2. Materials and Methods

2.1 Plant Collection and authentication

The fresh leaves of *R. nasutus* were collected from Tirumala Hills, Tirupati, Chittoor district of Andhra Pradesh, India and authenticated by professor P. Jayaraman, Botanist, Director, Plant anatomy research centre, Tambaram, Chennai, India in the month of May 2014 and registered as PARC/2014/2075.

2.2 Preparation of plant extracts

The leaves were thoroughly washed with de-chlorinated water, shade dried at room temperature (28 ± 2 ºC) for 5 - 8 days. The dried leaves of *R. nasutus* were powdered using Commercial electrical blender. The plant material was loaded in Soxhlet apparatus and was extracted with four different solvents namely petroleum ether, chloroform, ethyl acetate and methanol individually [13]. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of the plant varied with the solvents used. Standard stock solutions were prepared at 1% by dissolving the residues using the universal solvent DMSO (di-methyl sulphoxide). From this stock solution different concentrations (60-300 ppm) were prepared.

2.3 Culture of test organism

The eggs of *Culex quinquefasciatus* and *Aedes aegypti* were collected from zonal entomological team, Vellore, Tamil Nadu, India, using an "O"- type brush. These eggs were brought to the laboratory and transferred to (18×13×4 cm) enamel trays containing 500 ml of water for hatching. The mosquito larvae were fed with pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage. The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500ml of water with the help of a dipper. The plastic jars were kept in a (60×60×60 cm) mosquito cage for adult emergence. Mosquito larvae were maintained at 27 ± 2 °C, 75 – 85% relative humidity, under a photoperiod of 14:10 hrs light: dark. A 10% sugar solution was provided for a period of 3 days before blood feeding. The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days to ensure adequate blood feeding for 5 days.

2.4 Repellent activity

The repellent study was followed by the method of W.H.O. (2009) [14]. Three days old blood starved females of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes (100) were kept in a net cage (45 X 30 X 45 cm^2). The volunteer had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. The arm of volunteer, only 25 cm^2 dorsal side of the skin on each arm was exposed and the remaining area covered with rubber gloves. The leaf extracts were applied at 1.0, 2.0 and 3.0 mg/cm^2 separately in the exposed area of the forearm. Ethanol was served as the control. *Cx. quinquefasciatus* was tested during the night from 19.00 to 05.00 hours. *Ae. aegypti* was tested during the day time from 7.00 to 17.00 hours. The control and treated arm were introduced simultaneously into the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. The volunteer conducted test of each concentration by inserting the treated and control arm into the cages at a same time for one full minute for every 5 minutes. Until a confirmed bite was received the test was over after the conformation of mosquito bite in extract to be tested. The mosquito repellency of different extracts was measured on the basis of the protection time (min) the time was introduced simultaneously into the cage. The number of bites was counted over 5 minutes for every 30 minutes. The experiment was conducted six times. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula.

$$\% \text{ Repellency} = \frac{(Ta - Tb)}{Ta} * 100$$

Where
Ta – Number of mosquitoes in the control group
Tb – Number of mosquitoes in the treated group.

2.5 Adulticidal activity

Five to six day old sugar-fed adult female mosquitoes were used. *Rhinacanthus nasutus* leaf extracts were diluted with ethanol to make different concentrations. The diluted plants extracts were impregnated on filter papers (140*120 mm). A blank paper consisting of only ethanol was used as a control. The papers were left to dry at room temperature to evaporate off the ethanol overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes, both measuring 125*44 mm following the WHO method [15]. One tube served to expose the mosquitoes to the plant extracts and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16 mesh size wire screen. Sucrose-fed and blood starved mosquitoes (25) were released into the tube, and the mortality effects of the extracts were observed every 10 minutes for 3 hours exposure period. At the end of 1, 2, and 3 hrs exposure periods, the mosquitoes were placed in the holding tube. Cotton pads soaked in 10% sugar solution with vitamin-B complex were placed in the tube during the holding period of 24 hours and Mortality rate was recorded. The above procedure was carried out in triplicate for each concentration. Adulticidal activity was calculated by counting dead mosquito from the introduced mosquito. Any mosquito was considered to be dead if did not move when prodded repeatedly with a soft brush. The control mortalities were corrected by using Abbott's formula [16]. The LC_{50} and LC_{90} were calculated from toxicity data by using Probit analysis [17].

$$\text{Corrected Mortality} = \frac{\% \text{ Test Mortality} - \% \text{ Control Mortality}}{100 - \% \text{ Control mortality}} \times 100$$

2.6 Statistical analysis
The average adult mortality data were subjected to Probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the SPSS 20.0 version software. Results with $P \leq 0.05$ were considered to be statistically significant.

3. Results
3.1 Adulticidal Bioassay
The results of the adulticidal activity of petroleum ether, chloroform, ethyl acetate and methanol leaf extract of *R. Nasutus* against the adult of two important vector mosquitoes *Aedes aegypti* and *Culex quinquefasciatus* are presented in Figure 1. Among two vectors tested the highest adulticidal activity was observed in Methanol. At higher concentrations, the adult showed restless movement for sometimes with abnormal wagging and died. LC_{50} and LC_{90} values were calculated.

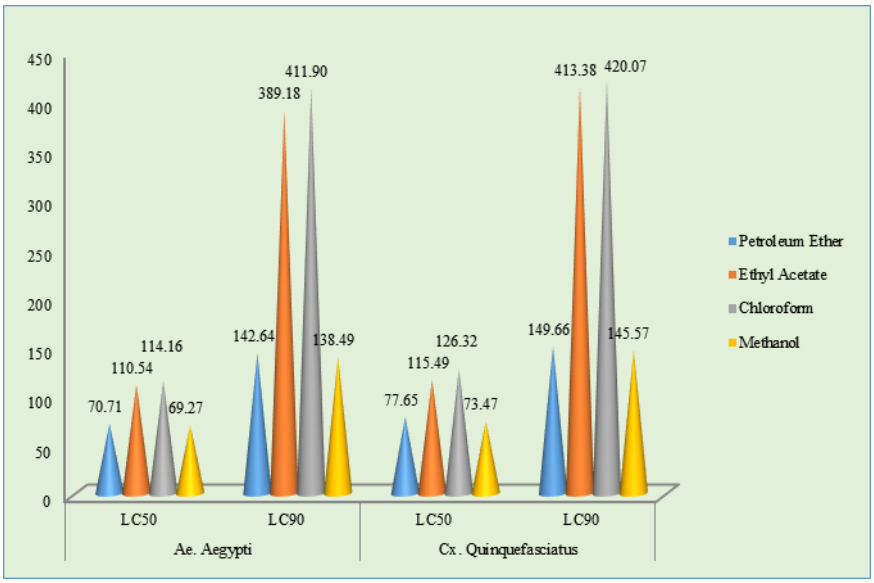

Fig 1: Adulticidal activity of *Rhinacanthus nasutus* leaf extracts against *Aedes aegypti* & *Culex quinquefasciatus*

3.2 Repellent Activity
The skin repellent activity of petroleum ether, chloroform, ethyl acetate and methanol extract of *Rhinacanthus Nasutus* against blood starved adult female of *Aedes aegypti* and *Culex quinquefasciatus* are given in Table 1 and 2. The Methanol extract had strong repellent action against mosquitoes as it provided 100% protection in all the concentrations against *Ae. aegypti* and *Cx. quinquefasciatus* for 210 min followed by a Petroleum ether extract which revealed repellent action for 180 min in both the mosquito species. It clearly shows that repellent activity was dose dependent. From the results we concluded that the leaf extract of *R. Nasutus* provided an excellent potential for controlling *Ae. aegypti* and *Cx. quinquefasciatus*.

Table 1: Repellent activity of *R. Nasutus* leaf extract against *Aedes Aegypti*

Extracts	Conc. mg/cm²	Percentage of repellency, Time post application of repellent(min)							
		30	60	90	120	150	180	210	240
Pet. Ether	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	97.3 ± 1.6	79.3 ± 0.8
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	83.2 ± 1.2
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	91.3 ± 1.6
Chloroform	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	98.7 ± 0.8	78.3 ± 1.6	67.7 ± 1.9	56.5 ± 1.8
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	83.3 ± 1.6	73.3 ± 1.5	61.0 ± 1.4
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.2 ± 1.5	82.0 ± 1.4	73.3 ± 1.9
E. Acetate	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	81.2 ± 1.9	72.3 ± 1.9	59.7 ± 1.6
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	87.5 ± 1.6	79.7 ± 1.8	64.2 ± 1.3
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	97.3 ± 1.0	86.2 ± 1.6	77.3 ± 1.9
Methanol	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	81.5 ± 1.1
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	85.7 ± 1.4
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	98.1 ± 1.6

Table 2: Repellent activity of R. Nasutus leaf extract against Culex quinquefasciatus

Extracts	Conc. mg/cm^2	Percentage of repellency, Time post application of repellent(min)							
		30	60	90	120	150	180	210	240
Pet. Ether	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.4 ± 1.3	78.7 ± 1.5
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	81.3 ± 0.8
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	87.7 ± 1.4
Chloroform	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	98.0 ± 1.3	76.2 ± 1.6	67.3 ± 1.6	54.2 ± 1.7
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	81.7 ± 1.5	73.3 ± 1.0	60.7 ± 1.2
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	92.3 ± 1.9	80.2 ± 1.7	71.3 ± 1.5
E. Acetate	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	80.3 ± 1.5	70.8 ± 1.0	58.7 ± 1.4
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	85.8 ± 1.8	77.3 ± 1.9	63.2 ± 1.3
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.0 ± 1.3	84.8 ± 1.2	75.7 ± 1.2
Methanol	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	80.2 ± 0.8
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	84.5 ± 1.1
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.2 ± 1.3

4. Discussion

In our results showed that leaf extract of *Rhinacanthus nasutus* having significant adulticidal and repellent activity against *Aedes aegypti* and *Culex quinquefasciatus*. The results are comparable with the earlier references. Our previous study revealed adulticidal mortality was highly significant in the petroleum ether extract of *Justicia adhatoda* leaf showed LC_{50} values of 73.50, 72.14 ppm and LC_{90} values of 142.02, 225.07 in *Culex quinquefasciatus* and *Aedes aegypti*. The petroleum ether extract had strong repellent action against mosquitoes as it provided 100% protection against *Culex quinquefasciatus* 180 min followed by *Aedes aegypti* for 210 min [18]. The ethanol extract of *Andrographis paniculata* exhibited highest adulticidal activity of 94.2% of mortality at 3.0 mg/cm^2 [19]. The highest adult mortality was found in the methanol extract of *Andrographis paniculata* against the adults of *Culex quinquefasciatus* and *Aedes aegypti* with the LC_{50} and LC_{90} values were 149.81, 172.37 ppm and 288.12, 321.01 ppm respectively [20]. The maximum efficacy was observed in the methanol extract. The LC_{50} and LC_{90} values of *Eclipta alba* and *Andrographis paniculata* against adults of *Anopheles stephensi* were 150.36, 130.19 ppm and 285.22, 244.16 ppm, respectively. Methanol extract of *Eclipta alba* and *Andrographis paniculata* was produce maximum repellency against *Anopheles Stephensi* [21]. Adulticidal activity, the LC_{50} and LC_{90} values of hexane, dichloromethane, ethyl acetate and methanol extracts of *Barleria prionitis* against *Culex quinquefasciatus* larvae in 24h were 280.25, 269.21, 253.12, 237.4 and 484.56, 473.39, 459.81 and 450.82 ppm, respectively [22]. The maximum repellent activity was observed at 500 ppm in methanol extracts of *Aegle marmelos*, *Acacia lineata*, and ethyl acetate extract of *Chamaecytisus hirsutus*, and the mean complete protection time ranged from 90 to 120 min against *Anopheles subpictus*. The hexane extract of *Andrographis paniculata* was more effective in exhibiting the repellent action against the mosquito as compared with *Acacia lineate* extract and the complete protections was observed for 150 min in the hexane extract of *Andrographis paniculata* at 500 ppm against *Culex tritaeniorhynchus* bites [23]. The skin repellent test at 1.0, 2.5, and 5.0 mg/cm^2 concentration gave the mean complete protection time ranged from 119.17 to 387.83 min against *Anopheles stephensi* with the benzene, petroleum ether, ethyl acetate, and methanol extracts of *Citrullus vulgaris* tested [24]. The *Loranthus pentandrus* methanol leaf extract had strong repellent action against *Aedes aegypti* mosquito as it provided 100% protection against 240 min at 4 and 5 mg/cm^2 followed by ethyl acetate, dichloromethane, diethyl ether and hexane [25]. The methanol extract of *calotropis procera* extract had strong repellent action against selected species of mosquitoes as it provided 100% protection against *Aedes aegypti* for 210 min followed by *Anopheles stephensi* (180 min) and *Culex quinquefasciatus* (150 min) at 1.0, 2.0 and 3.0 mg/cm^2 [26]. The adult mortality was observed in methanol extract. The LC_{50} and LC_{90} values of *Cassia tora* leaf extracts against adulticidal activity of (hexane, chloroform benzene, acetone, and methanol) *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* were the following: *Culex quinquefasciatus* LC_{50} values were 338.81, 315.73, 296.13, 279.23 and 261.03 ppm and LC_{90} values were 575.77, 539.31, 513.99, 497.06 and 476.03 ppm; *Aedes aegypti* LC_{50} values were 329.82, 307.31, 287.15, 269.57 and 252.03 ppm and LC_{90} values were 563.24, 528.33, 496.92, 477.61 and 448.05 ppm; and *Anopheles stephensi* LC_{50} values were 317.28, 300.30, 277.51, 263.35 and 251.43 ppm and LC_{90} values were 538.22, 512.90, 483.78, 461.08 and 430.70 ppm respectively. The results of the repellent activity of methanol extracts of *Cassia tora* plant at three different concentrations of 1.0, 2.5, and 5.0 mg/cm2 ranged at 210 min [27]. The adulticidal activity of hexane, ethyl acetate, benzene, chloroform and methanol leaf and seed extract of *Pithecellobium dulce* against *Culex quinquefasciatus*. The LC_{50} and LC_{90} values of leaf and seed methanol extracts of *Pithecellobium dulce* against *Culex quinquefasciatus* were 234.97, 309.24 ppm and 464.86, 570.80 ppm respectively [28]. The mosquito sensitivity to repellents varies among *Aedes, Anopheles* and *Culex* mosquitoes [29]. A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors, but very few plant products have shown practical utility for mosquito control. Some of the plants that have been tested against mosquito larvae in India are *Cleome viscosa*, *Ocimum basilicum*, *Vitex negundo*, *Delonix regia*, *Oligo chaetaramosa*, *Azadirachta indica*, *Quassia amara*, *Anacardium occidentale*, *Thevetia Neriifolia*, etc. Natural products are preferred because of their bio-degradability and lesser toxicity compared to the synthetic ones [30]. Compared with earlier references our results revealed that the experimental plant extracts were effective to control *Culex quinquefasciatus* and *Aedes aegypti*.

The finding of the present investigation revealed that the crude extract of *Rhinacanthus nasutus* possesses remarkable adulticidal and repellent activity against medically two important vector mosquitoes.

5. Conclusion

Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive components and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals for control of insect vectors.

6. Competing Interest

The authors declare that they have no competing interests.

7. Acknowledgement

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