RESEARCH

27(96), January 1, 2015

ISSN 2278–5469 EISSN 2278–5450

Discovery

Mosquito adulticidal and repellent activities of *Loranthes pentandrus* against dengue vector, *Aedes aegypti* (Diptera : Culicidae)

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Publication History

Received: 07 October 2014

Accepted: 06 December 2014

Published: 1 January 2015

Citation

Krishnappa K, Elumalai K. Mosquito adulticidal and repellent activities of *Loranthes pentandrus* against dengue vector, *Aedes aegypti* (Diptera : Culicidae). *Discovery*, 2015, 27(96), 8-14

ABSTRACT

To determine the adulticidal and repellent activities of different extracts of *Loranthes pentandrus* against *Aedes aegypti*. Ten pair of selected adult mosquito were exposed to various concentrations (100-500ppm) and were assayed in the laboratory by using the protocol of WHO 2005; the 24 h LC_{50} values of the *Loranthes pentandrus* leaf extract was determined following Probit analysis. The repellent efficacy was determined against selected mosquito species at five concentrations (1-5 mg/cm^2) under the laboratory conditions. The significant adulticidal activity was recorded from the highest concentration of methanol extract at 500 ppm and the least larvicidal activity was recorded from the 100ppm concentration of Diethyl ether extract. Lethal concentration, the LC_{50} and LC_{90} values of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts of *Loranthes pentandrus* against *Aedes aegypti* adult in 24 h were 267.80, 269.65, 263.11, 254.79 and 248.57 479.04, 468.03, 463.03, 441.72, and 432.75ppm, respectively. The *Loranthes 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. From the results it can be concluded the leaf extract of *Loranthes pentandrus* was an excellent potential for controlling *Aedes aegypti* mosquito.

Key Words: Adulticidal activity, Repellent activity, *Loranthes pentandrus, Aedes aegypti*.

1. INTRODUCTION

Vector-borne diseases are infectious diseases that are transmitted by organisms that include insects, snails and rodents. These diseases represent a heavy burden on people, their families and communities in developing countries. Some of the most debilitating of these diseases are malaria, dengue, lymphatic filariasis, Japanese encephalitis, leishmaniasis, onchocerciasis, schistosomiasis and trypanosomiasis. For example, lymphatic filariasis can cause morbidity for life, while malaria causes the highest mortality, especially among young children and pregnant women. Vector-borne diseases also result in school absenteeism, loss of productivity, aggravation of poverty, high costs for health care and a burden on public health services (WHO, 2012). *Ae. aegypti* Linn. (Diptera: Culicidae) is the most important vector of dengue viruses world-wide, yellow fever virus in urban settings, and is a competent vector of chikungunya virus. Dengue causes more human morbidity and mortality than any other vector-borne viral infection. *Ae. aegypti* is uniquely adapted to a close association with humans, which facilitates efficient virus transmission (Morrison et al, 2008). This mosquito is more widely dispersed now than any time in the past, placing billions of humans at risk of infection. It enjoys greater geographical distribution and is established virtually in all tropical countries (Halstead, 2008). In the absence of an effective vaccine/antiviral therapy, vector control is at present the only way to limit these mosquito-borne diseases (Mariappan, 2007). Conventional pesticides such as malathian, DDT and pyrethroides that are generally used for mosquito control are known to cause the problems such as environmental pollution, residual effects and resistance of mosquito species. Development of resistance in *Ae. aegypti* has been noted by World Health Organization, (2010) and by other studies (Raghavendra et al, 2011). Resistance to insecticides is an increasing problem in vector control because of the reliance on chemical control and expanding operations, particularly for malaria and dengue control. Furthermore, the chemical insecticides used can have adverse effects on health and the environment. Vector control is often not sufficiently adapted to local or changing circumstances because many countries lack capacity in decision-making for vector control. Such decisions should be based on evidence about the characteristics of local vectors and human behaviour and on the effectiveness of vector control methods. Furthermore, aspects of global change, such as climate change, environmental degradation, water scarcity and urbanization, are affecting the distribution of vector-borne diseases. Vector control must be adapted locally to these diverse and changing conditions and also to community preferences and needs. These problems forced to search for new, alternative and safer control measures especially from plant source. Because, plant derived molecules are eco-friendly, biodegradable and target specific (Nathan and Kalaivani, 2005).

Moreover, the development of resistance by vectors against plant derived molecules has not been reported so far. This has necessitated the need for search and development of environmentally safer, low cost, indigenous methods for vector control. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Promsiri et al, 2006). In addition to application as general toxicant against mosquito larvae, botanical insecticides also have potential uses as growth and reproduction inhibitors, repellents, ovicidal and oviposition deterrents (Prajapati et al, 2005). A huge number of botanical derivatives exhibited mosquitocidal activity (Youssif and Shaalan, 2011). The bioactive constituents of these plants could be either a single substance or a mixture of substances. The separation of the mixture is neither practical nor advantageous in the insect economic control strategies. The aim of the current study is to investigate the chemical composition and mosquito repellent and adulticidal activity of *Loranthes pentandrus* medicinal plant against dengue vector mosquito *Ae. aegypti* (Diptera : Culicidae).

2. MATERIAL AND METHODS

2.1. Plants collection and solvent extraction

Matured leaves of *Loranthes pentandrus* Linn. (Loranthaceae) were collected during the flowering season (October 2013 - December 2013) in and around Yercaud hill station (11.77940N, 78.20340E) Salem District of the Tamilnadu India. The collected leaves brought to the laboratory where, they were washed thoroughly with tap water and kept in sunlight for 45 minutes for the complete evaporation of water and then shade dried on blotting paper spread at room temperature (28 ± 2 °C). The dried plant material was powdered using electric blender and extracted with selected solvent using Soxhlet apparatus. The solvent from the crude extract was

evaporated using rotary evaporator at 45ºC until the complete evaporation of solvent, the crude extract was weighed and stored in an aseptic amber bottle vials at 4ºC in the refrigerator.

2.2. Extraction

The leaves of *Loranthes pentandrus* were washed with tap water, shade-dried, and finely ground with the help of electrical blender. The finely ground plant leaf powder (1.0 kg) was loaded in Soxhlet apparatus and was extracted with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol by adapting a standard protocol(Vogel, 1978). The solvents from the extracts were removed using a rotary vacuum evaporator to collect the crude extract. Standard stock solutions were prepared to 100, 200, 300,400 and 500ppm by dissolving the residues in their respective solvent.

2.3. Test organisms

The larvae of mosquito, *Ae. aegypti* were collected from the agricultural gardens and field and continuously reared in the laboratory. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week-old chick for blood meal. Mosquitoes were held at 27 ± 2°C, 75±5% RH, with a photo period of 12L: 12D.

2.4. Adulticidal activity

The adulticidal activity of the extracts was assessed by following the method of WHO (1981). Freshly moulted adult mosquitoes (0–24h old; fed with sugar with multivitamin and blood-starved) mosquito 30 nos. were collected from the insect rearing cage and gently transferred into a glass holding tube. The different concentrations (40-240 ppm) of the plant extracts were prepared in acetone and applied on filter papers (size 120×120mm). Control paper was treated with acetone only under similar conditions. The diluted plant extracts were impregnated on filter papers and the papers were left to dry at room temperature to evaporate off the acetone overnight. Impregnated papers were filter papers (size 120×120mm). Control paper was treated with acetone only under similar conditions. The diluted plant extracts were impregnated on filter papers and the papers were left to dry at room temperature to evaporate off the acetone overnight. Impregnated papers were prepared afresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical glass tubes both measuring 100ml. 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 12 mesh size cotton cloth. The adult mosquitoes were released into the tube, and the mortality was observed every 15min for 3 h exposure period. At the end of 1, 2, and 3 h exposure periods, the mosquitoes were placed in the holding tube. The above experiment was carried out in triplicate for each test 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.

2.5. Repellent Activity

The repellent study will be made by following the method of WHO, (2005). Three-day-old blood-starved female mosquitoes (100) were kept in a net cage (45 cm × 30 cm × 45 cm). The unteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of unteer, only 25 cm^2 dorsal side of the skin on each arms were exposed and the remaining area covered by rubber gloves. The crude extract was applied at 1.5, 3.0 and 4.5 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. The time of the test dependent on whether the target mosquitoes day-or night biters. *Ae.aegypti* will be tested during the day time from 07.00 to 17.00h, while *Cx. quinquefasciatus* and *An. stephensi* will be tested during the night from 19.00 to 05.00h. The control and treated arm will be introduced simultaneously in to the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes will be activated. Each test concentration was repeated six times. The unteer conducted their test of each concentrations were inserted their treated and control arm in to the same cage for one full minute for every five minutes. The mosquitoes that landed on the hand will be recorded and then shaken off before imbibing any blood; making out a 5 minutes protection. The percentage of repellency will be calculated by the following formula.

$$\% \text{ Repellency} = [(T_a - T_b)/T_a] \times 100$$

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

2.6. Statistical Analysis

The average mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics chi-square, Slope, Regression values were calculated by using the software using statistical package of social science (SPSS) version 18.0 for windows, significance level was set at $P<0.05$.

3. RESULTS AND DISCUSSION

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide must not cause high mortality in target organisms in order to be acceptable many researchers. The results of the present study clearly have shown in table 1 and 2. Data of the adulticidal activity of leaf extract of *Loranthes pentandrus* against the *Aedes aegypti* are presented in Table 1. The significant adulticidal activity was recorded from the highest concentration of methanol extract at 500 ppm and the least larvicidal activity was recorded from the 100ppm concentration of diethyl ether extract. Lethal concentration, the LC_{50} and LC_{90} values of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts of *Loranthes pentandrus* against *Aedes aegypti* adult in 24 h were 267.80, 269.65, 263.11, 254.79 and 248.57 479.04, 468.03, 463.03, 441.72, and 432.75ppm, respectively. The *Loranthes 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 (Table 3). It showed that repellency depends on the strength of the extract concentration. From the results it can be concluded the leaf extract of *Loranthes pentandrus* was an excellent potential for controlling *Aedes aegypti,* mosquito.

Table 1

Adultiicidal activity of *Loranthes pentandrus* extracts tested against freshly emerged adults of *Aedes aegypti* mosquito

	Larval Mortality* (%), *Loranthes pentandrus* extracts				
Concentration (ppm)	**Hexane**	**Diethyl ether**	**Dichloromethane**	**Ethyl acetate**	**Methanol**
100	19.6±1.6 [b]	15.4±1.6[b]	18.4±1.4 [b]	19.3±1.3 [b]	20.4±1.6 [b]
200	30.2±1.4 [c]	31.2±1.2[c]	31.6±1.7 [c]	31.6±1.8 [c]	32.2±1.2 [c]
300	55.6±2.8 [d]	57.8±2.8[d]	56.4±2.2 [d]	59.4±2.6 [d]	60.6±2.4 [d]
400	76.2±2.2 [e]	76.6±2.2[e]	78.8±2.5 [e]	79.3±2.4 [e]	82.2±2.2 [e]
500	95.4±2.8 [f]	95.6±2.6[f]	96.6±2.2 [f]	100.0 ±0.0 [f]	100.0 ±0.0 [f]
Control	1.8±0.6 [a]	2.6±0.8 [a]	2.4±1.9 [a]	2.2±1.4 [a]	2.8±1.5 [a]

Value represents mean± S.D. of five replications. *Mortality of the adults observed after 24h of exposure period (WHO, 2005). Values in a column with a different superscript alphabet are significantly different at $P < 0.05$ (MANOVA; LSD -Tukey's Test).

Table 2

Leathal concentration of *Loranthes pentandrus* extracts tested against the freshly emerged adults of *Aedes aegypti*

Solvent tested	LC_{50}(ppm)	95% Fiducial Limit (ppm)		LC_{90} (ppm)	95% Fiducial Limit (ppm)		Slope	χ^2
		LCL	UCL		LCL	UCL		
Hexane	267.80	224.61	307.80	479.04	420.97	578.80	3.3932	4.135
Diethyl ether	269.65	249.31	289.29	468.03	436.89	508.57	3.6443	2.013
Dichloromethane	263.11	224.95	298.52	463.03	411.89	545.68	3.6258	3.565
Ethyl acetate	254.79	189.28	311.91	441.72	371.44	597.57	4.1490	9.392
Methanol	248.57	186.49	302.17	432.75	366.08	574.06	4.2558	8.619

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Though there are no reports available regarding the potential of *Loranthes pentandrus* as mosquito adulticidal and repellent activity, several reports are available on other plant extracts and atile oils which reveal their efficacy against mosquito larvae. The result of the present study was also comparable to the earlier reports, Liang Zhu and Ying Juan Tian, (2011) analysed the chemical composition of *Blumea martiniana* and assayed them for their larvicidal activity against *An. stephensi*. Cheng et al, (2004) compared the essential oils from the leaves of *Cinnamomum osmophloeum* had an excellent inhibitory effect against the fourth instar larvae of *Ae. aegypti*. The larvicidal activity of cinnamon and other oils were recorded by Zhu et al, (2008) against 4th instars of *Ae. albopictus, Ae. aegypti*, and *C. pipiens pallens*. Pushpalatha and Muthukrishnan, (1995) reported that the petroleum ether : ethyl acetate (3:1)

fraction of *V. negundo* leaf extract showed LC_{50} value of 8.21 ppm against the 2nd instar larvae of *C. quinquefasicatus*. But the 2nd instar larvae are more susceptible to larvicidal principles than the 4th instar larvae. A saponin isolated from *Achyranthus aspera* recorded the LC_{50} valueof 18.20 and 27.24 ppm against *A. aegypti* and *C. quinquefasciatus*, respectively (Bagavan et al, 2008).The LC_{50} values of Borneol were 43.5 mg/L against the larvae of *An. Aegypti* (Rajkumar and Jebanesan, 2010).Several researchers reported, phytochemical based experiments for exploring the insecticidal activity on mosquito vectors (Elumalai et al, 2012a; Elumalai et al, 2012b; Krishnappa et al, 2012; Krishnappa and K. Elumalai, 2012a; Krishnappa and Elumalai, 2012b; Krishnappa and K. Elumalai, 2013). A number of studies have also been carried out on the larvicidal potential of essential oil extracted from the Citrus leaves and peels (Melliou et al, 2009). Redwane et al, 2002 reported that gallotannins isolated from *Quercus lusitania* var.*infectoria* galls had the LC_{50} value of 373 ppm against *C. pipiens*. Essential oil of *Cinnamomum zeylanicum, Zingiber officinale* and *Rosmarinus officinalis* also showed repellent activities against *An. stephensi, Ae. aegypti* and *C. quinquefasciatus* (Gillij et al, 2008). Komalasmira et al, 2005 who have been reported the ethanol extracts of *P. beetle* has successfully killed the larvae of 4 mosquito vectors *Ae. aegypti, C. quinquefasciatus, An. dirus* and *Monsonia uniformis*. Mosquito control is vital for many countries and is still in a state of eution. During the last decades, it depended upon synthetic organic insecticides, many of which have been removed from the arsenal of weapons Floore, (2006) and botanicals are the new weapons of mosquito control under exploration. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. Natural pesticides derived from plants are a promising tool especially for targeting mosquitoes in the larval stage (Amer and Mehlhorn, 2006).

Table 3

Repellent activity of *Loranthes pentandrus* extracts tested against *Aedes aegypti* mosquito.

	% of Repellent activity Time post application of repellent (min)							
Concentration (µg/cm²)	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**	**210 min**	**240 min**
				Hexane				
1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	42.3 ±1.8	38.6±1.4	24.8±1.4
2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	58.4±1.5	41.8 ±1.2	31.2 ±1.6
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	62.4±1.7	52.4±2.8	46.8±2.8
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	74.8±2.6	69.7±2.8	52.2±2.5
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.6±2.2	71.4±3.6	66.2±2.4
				Diethyl ether				
1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	45.4±1.3	39.4±1.8	23.4±1.2
2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	58.6±1.4	46.6±1.2	34.2±1.6
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	62.7±2.9	51.2±2.4	39.2±2.8
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	70.2 ±2.4	63.8±2.3	42.4±2.6
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	79.3±2.6	67.6±2.8	48.3±2.4
				Dichloromethane				
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	51.2±2.2
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	69.7±2.8
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	73.9±2.5
4.0	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	79.8± 2.7
5.0	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	82.6±2.2
				Ethyl acetate				
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	68.2±2.4
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	71.8±2.1
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	78.2±2.1
4.0	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	82.2±2.6
5.0	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	88.4±2.3
				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	88.2±2.5
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	92.7±2.9

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	96.9±2.7
4.0	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	100.0±0.0
5.0	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	100.0±0.0

Value represents mean± S.D. of five replications.

Ansari et al, 2000 suggested that the peppermint oil (*Mentha piperita*) showed strong repellent activity against adult mosquitoes when applied on the human skin. The protection obtained against *An. annularis, An. culicifacies,* and *C. quinquefasciatus* was 100.0%, 92.3%, and 84.5%, respectively. Nathan et al, 2005 considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity against *An. stephensi* and the larval mortality was dose-dependent with the highest dose of 1 ppm azadirachtin, evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *An. stephensi*. Kamaraj et al, 2008 have reported that the peel methanol extract *C. sinensis*, leaf and flower ethyl acetate extracts of *O. canum* against larvae of *An. Stephensi* (LC_{50} 95.74, 101.53, 28.96; LC_{90} 303.20, 492.43 and 168.05 ppm) respectively. Similarly, the aqueous and hydro-alcoholic extracts of *Melia azedarach* leaves and seeds were tested to explore the in vitro ovicidal and larvicidal activity against *Haemonchus contortus* (Sharma, et al, 2006). Karunamoorthi and Ilango, 2010 have reported that the LC_{50} and LC_{90} values of methanol leaf extracts of *Croton macrostachyus* were 89.25 and 224.98 ppm, respectively against late third instar larvae of malaria vector, *An. arabiensis*. The screening of *Artimisia annua* plants against larvicidal activity of *Anopheles* mosquito, it produced maximum activity and LC_{50} values were 16.85 ppm and 11.45 ppm after 24 and 48 h of exposure, respectively (Singh et al, 2006). The larvicidal and adulticidal activities of ethanolic and water mixture (50:50) of plant extracts *Eucalyptus globulus, Cymbopogan citratus, Artemisia annua, Justicia gendarussa, Myristica fragrans, Annona squamosa* and *Centella asiatica* were tested against *An. stephensi*, and the most effective between 80% and 100% was observed in all extracts (Senthilkumar et al, 2009). The biological activity of the plant extract might be due to a variety of compounds in *V. zizanioides* roots, including phenolics, terpenoids and alkaloids. These compounds may jointly or independently contribute to cause oviposition deterrent and ovicidal activity against *A. stephensi* (Medhi et al, 2010). The current study is to investigate the chemical composition and mosquito repellent and adulticidal activity of *Loranthes pentandrus* medicinal plant against dengue vector mosquito *Ae. Aegypti*.

4. CONCLUSION

In the present investigation has been under taken to establish the possible role of *Loranthes pentandrus* medicinal plant to control mosquito. Natural products are generally preferred due to their least hazardous impact on non-target organisms and their innate biodegradability in Vector Control Programme (VCP). Application of synthetic mosquitocides caused several unwanted consequences such as allergic reactions and side effects. Thus, it paves the way for further exploration of phytopesticide that are easily available in nature and also environmentally safer to non-target organisms like mammals. The results reported in this study open the possibility for further investigations of the efficacy of adulticidal activity and repellent activity of natural product extracts as a potential agent for combating malarial vector mosquito *Ae. aegypti*.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ACKNOWLEDGEMENTS

Authors are gratefully acknowledged to Professor N. Kalaichelvi, Head, Department of Zoology, Principal, Govt. Arts College (Autonomous) Nandanam for their support and laboratory facilities provided, and Department of Science & Technology-Fast Track Young Scientist Project (DST, New Delhi) Ref. NO. SB/FT/LS-356/2012.

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