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ORIGINAL ARTICLE

Larvicidal efficacy of *Boerhavia erecta* (L) leaf extracts against Zika virus, malaria and Japanese encephalitis mosquito vectors

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ABSTRACT
Scientists are welcome to use natural insecticides at the early stages of insects not in later ones. In the past, vector mosquitoes and other domestic agricultural pests were eradicated using plants and their derivatives. In the present investigation, larvicidal efficacy of Boerhavia erecta leaves solvent crude extracts were assayed on larvae of Aedes albopictus, Anopheles subpictus and Culex tritaeniorhynchus mosquitoes under controlled laboratory conditions. Different concentrations, five replicates were set up and an equal number of control groups were set up at the same time. The dead larvae were counted after 24 h of treatment. Mosquitoes larvae were susceptible to all crude extracts (Ethyl acetate, Chloroform and Methanol) and 100% mortality observed in Ae. albopictus larvae. Compared to the ethyl acetate and chloroform crude extracts, methanol crude extract has excellent larvicidal ability on Ae. albopictus, An. subpictus and Cx. tritaeniorhynchus mosquitoes with low LC_{50} values were 31.93, 33.79 and 35.42 µg/ml, respectively. This study confirmed B. erecta as a promising natural insecticide particularly as larvicidal due to the low LC_{50} values.
Keywords: *Public health; Boerhavia erecta; Crude extracts; Phytochemicals; Larvicidal; Biotoxicity.*

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INTRODUCTION

Mosquitoes are the crucial vector for transmitting many arbovirus diseases and causing a nuisance in public [1]. The World Health Organisation reports that mosquito-borne diseases cause millions of deaths along with nearly a billion cases yearly [2]. These include the most common ones, dengue, Zika, chikungunya, yellow fever, filaria, Japanese encephalitis, West Nile Virus and malaria, which are transmitted by infected *Aedes, Anopheles* and *Culex* mosquitoes and cause serious health problems worldwide, particularly in tropical and subtropical areas [3]. Zika virus (ZIKV) infection is transmitted by *Aedes* genus mosquitoes, especially *Ae. albopictus* and can be maintained in sylvatic cycles, between mosquitoes and wild animals. In 2016, a case of congenital ZIKV was reported to have sensorineural hearing loss, suggesting a potential infant consequence other than microcephaly [4]. Worldwide, malaria caused around 1.2 million deaths in a single year in 2010 including deaths of children and adults [5]. Japanese encephalitis virus (JEV), is an extremely dangerous flavivirus that is spread by mosquitoes and causes encephalitis in both humans and horses [6]. In Southeast Asia and Western Pacific Regions, JEV transmission is endemic in 24 countries, representing over 3 billion individuals at risk of infection [7]. Most of the cases occur in children under the age of 15 and the illness has no specific treatment.

Mosquito management is considered an essential strategy for combating this problem because vaccinations are not available for most of these diseases. We mostly rely on the use of synthetic and commercial chemicals, including carbamates, organochlorines, organophosphates, Dichloro Diphenyl Trichloroethane (DDT) and others, for the control of mosquitoes [8]. Long-term application of synthetic

chemical insecticides for vector control has been linked to resistance development and adverse ecological and human impacts. Natural materials are the best substitute because they are safe, bio-sourced, environmentally friendly, and less harmful to non-target species and the ecosystem. Currently, the development of novel insecticides has focused more on eco-friendly and botanical-based pesticides [9].
Plants are known to develop secondary metabolites that protect them from diseases or insect pests [10]. Secondary metabolites from plants are essential as anti-nutritional substances in food and animal feed. Other secondary metabolites, including terpenoids, help plants communicate with other plants, attract pollinators, and protect from harmful insects. These general features suggest that natural pesticides derived from secondary metabolites in plants might be more crucial for vector control compared to the current control methods [11]. Many scientists throughout the world have documented the effectiveness of using various plant parts and products that are easily available in the area to control mosquitoes. Additionally, many compounds and extracts from different plants have been studied as potential novel larvicides [12;13;14].
Boerhavia erecta L. (Nyctaginaceae) is an erect, annual-to-perennial plant commonly known as the erect spiderling. The tropical regions of Asia and Africa, genus *Boerhavia* contain more than 100 species [15]. The entire plant has been used traditionally to cure a variety of illnesses, such as fungal infections, paediatric convulsions, hepatic, gastrointestinal, and reproductive issues [16]. It is discovered to contain alkaloids, flavonoids, saponins, steroids, and glycosides, in addition to more polar substances including proteins, minerals, sugar and vitamins [17]. Numerous natural products and synthesis compounds derived from *B. erecta* have demonstrated potent anticancer and antioxidant properties [18], antidiabetic and antihyperlipidemic activity [19], Cytotoxicity, Antimicrobial activity [20,21]. Based on the literature study and the need for an ecologically sustainable way to manage mosquito control, the current study was carried out larvicidal efficacy of ethyl acetate, chloroform and methanolic extracts of *B. erecta* evaluated against Zika virus vector *Ae. albopictus*, malaria vector *An. subpictus* and Japanese encephalitis vector *Cx. tritaeniorhynchus*.

MATERIALS AND METHOD

Chemicals
Ethyl acetate, chloroform and methanol were brought by Merck company, India. Required glassware had been thoroughly cleaned with chromic acid and sterilized with Millipore Milli-Q water.
Sample collection and processing
Healthy leaves of *Boerhavia erecta* were collected from the surrounding of Kumbakonam region, Thanjavur District, Tamil Nadu. After the leaves were collected, the dust particles on their surface were removed by washing them with tap water followed by distilled water. Washed leaves were allowed to shade dry at laboratory conditions (28 ± 2 °C) for five to eight days, or until they were readily broken by hand. After the leaf material was totally dry, a blender mixer was used to grind it into a fine powder (Fig. 1). The leaf powder was put in a dry jar to be used in future studies.

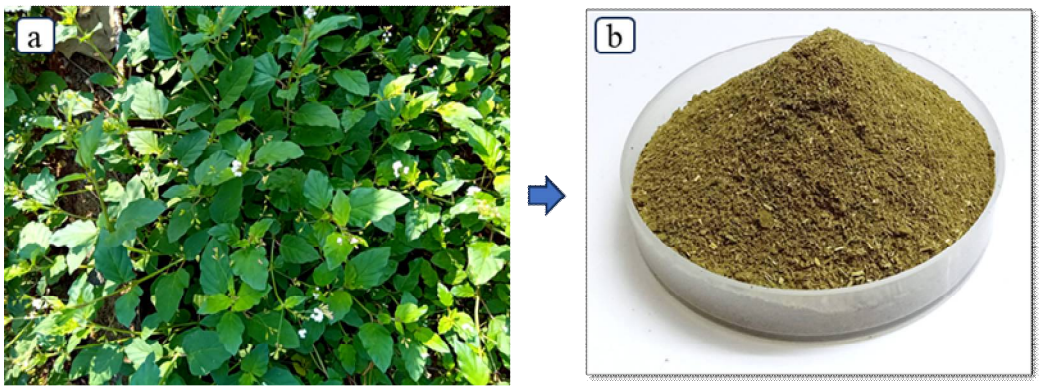

Fig. 1. a) *Boerhavia erecta* L. b) Leaf Powder

Extraction process
One liter of ethyl acetate, chloroform and methanol solvents were separately used for the extraction of 250 gm in the Soxhlet apparatus followed by Vogel, [22] method with slight modification. The powdered plant materials were put into the Soxhlet apparatus inner tube, and then placed into a circular bottom containing the appropriate solvents. Over a heating mantle, the solvent was gradually brought to a boil at

40 °C using an adjustable rheostat. The process was continued until complete extraction. The solvent was removed at the lower pressure using a rotary vacuum evaporator to get a viscous dark green residue of each solvent of ethyl acetate (13.5 g), chloroform (13.8 g) and methanol (14.2) leaf extracts (Fig. 2).

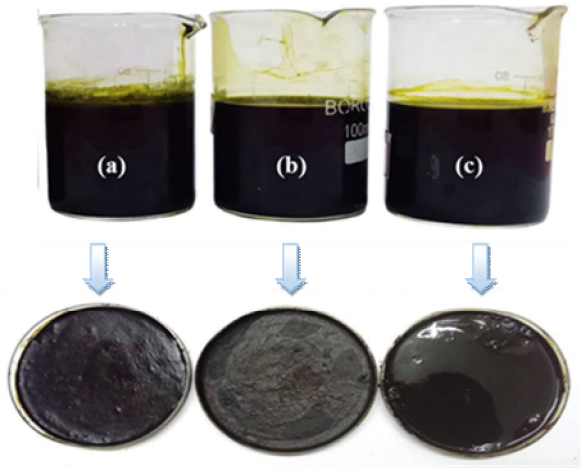

Fig. 2. a) Ethyl acetate crude extract, b) Chloroform crude extract, c) Methanol crude extract

Mosquito collection and rearing

Experimental bioassay was performed with 3rd instar larvae of *Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus.* These mosquito species eggs were identified and gathered from the Vector Control Research Centre (VCRC) in Pondicherry, India. Using the standard method developed by Govindarajan et al. [23], eggs were maintained and reared in our research laboratory (Centre for Animal Studies) Department of Zoology, Government College for Women (Autonomous), Kumbakonam, Tamil Nadu, India. After egg hatching, larvae were fed a 3:1 ratio of powdered dog biscuits and yeast tablets mixtures. The female adult mosquitoes were fed a blood meal via a membrane feeder, while the male adult mosquitoes were fed honey and a 5.0% glucose solution. Mosquitoes were kept at 28 ± 2 ℃, 70 to 85% relative humidity, with a photo period of 12-h light and 12-h dark.

Bioassay procedure

Assess the larvicidal efficacy of *B. erecta* crude extracts, an experimental bioassay was set up as outlined in the World Health Organisation guidelines [24] under controlled laboratory conditions. For the laboratory bioassay, various concentrations were tested. Methanol extracts 15, 30, 45, 60 and 75 µg/mL, chloroform extract 20, 40, 60, 80 and 100 µg/mL and ethyl acetate extract 25, 50, 75, 100 and 125 µg/mL. 25 late third-instar larvae of *Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus* were separately transferred to a 250-mL water cup containing 200 mL of dechlorinated water mixed with appropriate concentrations of solvent crude extract. For every concentration, five replicates were set up and an equal number of control groups were set up using tap water at the same time. The dead larvae were counted after 24 h treatment. Probit analysis was used to calculate LC_{50} and LC_{90} values [25].

Biotoxicity test on non-targeted species

For the biotoxicity test on non-target species, followed the method of Sivagnaname and Kalyanasundaram [26]. Crude extracts from *B. erecta* were evaluated for their biotoxicity to the aquatic non-target fish *G. affinis.* After their collecting from natural habitats, the specimens of this species were kept apart in cement tanks of 85 cm in diameter and 30 cm in depth, which were filled with water at a temperature of 27 ± 3°C and 85% relative humidity. The concentration of *B. erecta* solvent crude extracts was assessed at a level fifty times higher than the LC_{50} doses for mosquito larvae. 10 replicates of each concentration will be tested, and four replicates of the untreated control samples will also be included. The non-target species have been examined for anomalies, such as decreased activity, poor swimming ability, and mortality, after a 48-hour treatment period. Following treatment, we also studied the non-target species that were exposed to this extract every day for ten days in order to understand its impacts on swimming activity and survival.

Statistical analysis

Assessing biotoxicity on non-target organisms, the following formula [27]. was used to determine each non-target organism's Suitability Index (SI).

$$SI = \frac{LC_{50} \text{ of non-target organisms}}{LC_{50} \text{ of target vector species}}$$

Statistical evaluation was done using SPSS version – 26. Significance level was set at $p < 0.05$.

RESULTS

Larvicidal bioassay

Ethyl acetate, chloroform and methanol crude extracts of *B. erecta* were tested on *Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus* third instar larvae. In this experiment highest larval mortality was observed in *B. erecta* methanol crude extract on every mosquito species with low LC_{50} and LC_{90} values were 31.93, 33.79, 35.42 and 60.94, 63.45, 65.52 µg/mL, respectively (Table 1). In methanol crude extract *Ae. albopictus* mosquito larvae exhibited 100% mortality. Chloroform crude extract from *B. erecta* showed moderate larval mortality with LC_{50} values were 41.70, 44.03, 46.98 and LC_{90} values were 82.18, 87.06 and 91.41 µg/mL, respectively (Table 2) and 100% larval mortality observed on the *Ae. albopictus*. Ethyl acetate crude extract also exhibited moderate larval mortality with LC_{50} values of 54.29, 57.70, 61.12 and LC_{90} values of 103.51, 109.73 and 115.31 µg/mL respectively (Table 3). Followed by the methanol and chloroform extracts ethyl acetate also showed 100% mortality on *Ae. albopictus* larvae.

Biotoxicity on non-target species

In this investigation, the non-toxicity assessment of *B. erecta* leaf solvent crude extracts was assessed against the mosquito predatory fish *G. affinis*. After 48 h exposure, methanol, chloroform and ethyl acetate crude extract revealed no toxic effect on *G. affinis* with LC_{50} values 7440.02, 10853.37, 15485.82 and LC_{90} values 14449.19, 21756.02 and 29903.08 µg/mL, respectively (Table 4). SI revealed that *B. erecta* extracts were less harmful effect on non-target species *G. affinis* if compared to the targeted mosquito larval populations (Table 5).

Table 1: Efficacy of methanol crude extract of *Boerhavia erecta* against larvae of mosquito vectors.

Vector species	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	Regression equation	χ^2
Ae. albopictus	31.93 (28.58 – 34.93)	60.94 (56.62 – 66.58)	y= 8.40+ 1.264x	5.359*
An. subpictus	33.79 (30.49 – 36.80)	63.45 (59.00 – 69.28)	y= 5.20+ 1.285x	4.574*
Cx.tritaeniorhynchus	35.42 (32.15 – 38.43)	65.52 (60.96 – 71.50)	y= 2.56+ 1.301x	3.884*

Table 2: Efficacy of chloroform crude extract of *Boerhavia erecta* against larvae of mosquito vectors.

Vector species	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	Regression equation	χ^2
Ae. albopictus	41.70 (36.98 – 45.89)	82.18 (76.19 – 90.06)	y= 10.80+ 0.916x	5.625*
An. subpictus	44.03 (39.18 – 48.35)	87.06 (80.64 – 95.57)	y= 8.80+ 0.912x	2.270*
Cx.tritaeniorhynchus	46.98 (42.17 – 51.34)	91.41 (84.65 – 100.40)	y= 5.76+ 0.920x	0.811*

Table 3: Efficacy of ethyl acetate crude extract of *Boerhavia erecta* against larvae of mosquito vectors.

Vector species	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	Regression equation	χ^2
Ae. albopictus	54.29 (48.68 – 59.34)	103.51 (96.18 – 113.12)	y= 7.60+ 0.758x	5.651*
An. subpictus	57.70 (51.99 – 62.90)	109.73 (101.89 – 120.04)	y= 5.04+ 0.758x	2.572*
Cx.tritaeniorhynchus	61.12 (55.36 – 66.43)	115.31 (107.01 – 126.28)	y= 2.64+ 0.758x	0.506*

Table 4: Biotoxicity of solvent crude extracts of *Boerhavia erecta* against non-target organism *G. affinis*.

Test materials	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	Regression equation	χ^2
Methanol extract	7440.02 (6630.56 - 8161.90)	14449.19 (13410.07 - 15813.76)	y= 9.38+ 0.005x	5.483*
Chloroform extract	10853.37 (9613.12 - 11951.80)	21756.02 (20134.87 - 23909.50)	y= 9.93+ 0.003x	1.718*
Ethyl acetate extract	15485.82 (13864.71 - 16945.05)	29903.08 (27750.58 - 32734.89)	y= 7.40+ 0.002x	3.306*

*p≤0.05, Level of Significance.

Table 5: Suitability index of *G. affinis* over mosquito larvae exposed to *B. erecta* solvent crude extracts.

Test materials	*Ae. albopictus*	*An. subpictus*	*Cx. tritaeniorhynchus*
Ethyl acetate crude extract	285.24	268.38	253.36
Chloroform crude extract	260.27	246.49	231.02
Methanol crude extract	233.01	220.18	210.05

DISCUSSION

Tropical and sub-tropical regions of the world are facing continued rise in vector borne diseases. Use of chemical-based control measures to control mosquito nuisance has not only affected environment, bus also caused insecticide resistance in the mosquitoes and lethal effects on humans and non-target species. Mosquito larvicides derived from natural bio sources, particularly plants are effective and alternative for mosquito management [28]. As mentioned earlier, Asmaey et al. [29] demonstrated methanol extract from medicinal plants *Rosmarinus officinalis*, *Melissa officinalis* revealed excellent larvicidal ability on *Cx. pipiens* third instar larvae with LC_{50} values were 9.795 and 26.505 µg/mL, respectively.

Hari and Mathew, [30] have denoted the larvicidal ability of methanol, chloroform, and petroleum ether extract of *Lantana camara, Hyptis suaveolens*, *Tecoma stans and Nerium oleander* tested on *Ae. aegypti* and *Cx. quinquefasciatus* larva. Among these extracts, the petroleum ether extract of *L. camara* showed highest mortality with LC_{50} values were 10.63 mg/L followed by *T. stans* petroleum ether extract, *N. oleander* methanol extract, and petroleum ether extract of *H. suaveolens* with LC_{50} values were 19.26, 35.82, and 38.39 mg/L, respectively on the *Cx. quinquefasciatus*. In *Ae. aegypti*, *T. stans* petroleum ether extract showed the highest mortality with LC_{50} value was 55.41 mg/L followed by *H. suaveolens, L. camara* petroleum ether extract and *N. oleander* methanol extract with LC_{50} values were 64.49, 74.93 and 84.09 mg/L, respectively.

Acmella oleracea plant methanol, ethyl alcohol and hexane crude extracts resulted in maximum larvicidal activity on dengue vector *Ae. aegypti* along with low LC_{50} values were 23.2 µg/mL (Hexane), 28.42 µg/mL (Hydroethanolic) and 39.67 µg/mL (Methanolic) [31]. Maheswaran et al. [32] have reported *Couroupita guianensis* plant crude extract revealed no toxicity on non-target fish *Cyprinus carpio*. *Swietenia mahagoni* Jacq. methanolic extract exhibits low toxicity effect against non-target mosquito predator *Toxorhynchites splendens* [33].

CONCLUSION

In conclusion, our findings suggest that *B. erecta* may be an effective option for the long-term management of vector mosquitoes. Building on these results, scientists and professionals can endeavour to create more ecologically friendly and efficient strategies to manage mosquito populations and reduce the spread of illnesses like dengue, Zika virus, chikungunya, malaria, filariasis and encephalitis. This study provides foundations for further research initiatives that will enhance public health outcomes and encourage sustainable methods of disease prevention and control.

DECLARATION OF INTERESTS

The authors declare that they have no personal connections or competing financial interest.

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