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Control of human filarial vector, *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) through bioactive fraction of *Cayratia trifolia* leaf

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PEER REVIEW

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Comments

This research work has great commercial application in the field of vector control and human health and wellness. Although the authors used some old analytical techniques, still the outcome is very relevant. This outcome should be scale-up with industry collaboration in to market product.
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ABSTRACT

Objective: To investigate the mosquito larvicidal activity of *Cayratia trifolia* (L.) Domin (Vitaceae: Vitales) (*C. trifolia*) which is distributed in many parts of India with medicinal properties as vector control is facing threat due to the emergence of resistance to synthetic insecticides.
Methods: Young and mature leaves of *C. trifolia* were investigated for larvicidal activity against 3rd instars larvae of *Culex quinquefasciatus* in different seasons throughout the year. The active fractions were extracted using six different solvents in a non-polar to polar fashion *viz* petroleum-ether, benzene, chloroform: methanol (1:1 v/v), acetone, absolute alcohol and distilled water. Dose dependent mortality was recorded against each solvent extract. Determination of LD_{50} and LD_{90} were executed through log-probit analysis using the most bioactive fraction. The fluctuations in mortality were statistically co-related through ANOVA analyses concerning different seasons and types of leaves as random variables. Justification of larvicidal activity was established through student's *t*-test. Costing effects were evaluated on the non-target water fauna under laboratory conditions. Thin layer chromatographic techniques were performed for phytochemical analysis and categorization of chemical personality of the active fractions using the most effective solvent extract following standard methods.
Results: Significant variations in mortality rate were noted with respect to the type of leaves (mature and senescence), concentration of leaf extract and between seasons. The water extract among all the solvent extracts was found to induce cent percent mortality at 50 mg/L in test mosquito species within 24 h with a LD_{50} and LD_{90} value of 10.70 mg/L and 27.64 mg/L respectively. No significant mortality was recorded in non-target water population. Chromatographic analyses of the water extract revealed the presence of steroids, triterpene glycosides, essential oil, phenolics and diterpenes as secondary phytochemicals.
Conclusions: Water extract of *C. trifolia* leaf promised as a cost effective and potent larvicidal agent against *Culex quinquefasciatus*.

KEYWORDS
Cayratia trifolia, *Culex quinquefasciatus*, Larvicidal, Phytochemical analyses

1. Introduction

Synthetic pesticides have been used to reduce pest population since late sixties. Improper dosage and faulty application of synthetic insecticides result resurgence of secondary pest populations and increase resistance to disease vectors[1]. The search for new biodegradable insecticides having no ill effect on non target fauna remains the top priority[2]. Some phytochemicals have been identified to have good mosquito larvicidal properties[3-7]. For isolation of active principle from the plant parts different types of solvents, such as water, petroleum ether, chloroform and methanol are used. Tennyson *et al*[8] screened 150 plant species for their toxicities against

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mosquito and found several to be very effective.

Culex quinquefasciatus Say 1823 (*Cx. quinquefasciatus*) is the principal vector of bancroftian filariasis, and the eradication or control of vector, is regarded as one of the important alternative available in preventing and controlling filariasis[9]. Application of synthetic insecticides is, although, highly efficacious against the target species, vector control is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding vectorial capacity[10]. Furthermore, they are responsible for substantial hazards to a variety of non-target organisms and environment. The researchers therefore have diverted their attention towards plant kingdom, which are ecofriendly and cost effective.

During the present piece of study *Cayratia trifolia* (*C. trifolia*) leaves were selected on the basis of preliminary investigation on its mosquito larvicidal activity. Further it was subjected to solvent extraction to find out the most suitable solvent which inflects highest larval mortality. Chemical profile of the leaf extract was determined qualitatively. This is to mention that in traditional Indian medicine, leaves, seeds and roots of *C. trifolia* are used as astringent as well as applied to ulcers and boils. Leaves extract are diaphoretic which recommended in high fever. Roots have an anti-anaemic effect and all the aerial parts can activate CNS and have a hypothermic effect[11].

2. Materials and methods

2.1. Test mosquito larvae

The present study was conducted at Burdwan (23°16′ N, 87°54′ E), West Bengal, India during March 2012 to January 2013. Larvae of *Cx. quinquefasciatus* were collected from concrete drains surrounding the Burdwan University campus and kept in plastic buckets (15 L). The larvae were fed with powdered mixture of dog biscuits and yeast at 3:1 ratio and kept in a germ free condition, away from insecticides or repellants and maintained at (20±2) °C, 75%-85% RH, 14 L: 10D photoperiod cycles in an insectary. During the bioassay experiments, early 3rd instar larvae were taken from the bucket and transferred within the sterile glass dishes or beakers.

2.2. Description of the plant used

C. trifolia (L.) Domin (Vitaceae: Vitales) is a vine that climbs by means of tendrils. Leaves are trifoliate with petioles, 2 to 8 cm long, 1.5 to 5 cm wide, pointed at the tip and coarsely toothed at the margins. Flowers are small greenish white and borne on axillaries solitary cymes. Fruit is fleshy, juicy dark purple or black, subglobose and about 1 cm in diameter. Style short; stigma slightly or inconspicuously expanded. Berry globose shaped 1-4 seeded[12].

2.3. Preparation of crude extract of mature and immature leaves

Fresh, mature and immature leaves of *C. trifolia* were harvested separately from plants growing on outskirts of Burdwan. The plant was identified properly and a voucher specimen (ZGC-S-08) had been deposited at Botany Department, the University of Burdwan. After collection the leaves were initially washed with distilled water and dried on paper towel. Atotal of 50 g leaves were crushed with a Jankel and Kunkel model A10 mill, and the plant juice was filtered by Whatmans No. 1 filter paper and the clear filtrate was used as a stock solution (100% concentration of crude extract) for bioassay experiments. Required concentrations (0.6%, 0.5%, 0.4%, 0.3%, 0.2% and 0.1%) were prepared by mixing up of stock extract with appropriate quantity of sterilized distilled water.

2.4. Preparation of solvent extract of mature leaves

Fresh mature leaves were harvested, flushed with distilled water and dried in the shade at room temperature (20 °C) and crushed into fine powder with a Jankel and Kunkel model A10 mill. The dried leaf-powder (25 g) was put in a Soxhlet apparatus and the plant extracts were prepared according to Adhikari and Chandra[13] by using different 250 mL solvents of analytical grades (Merck) with gradually increasing polarity, *i.e.* petroleum-ether, benzene, chloroform: methanol (1:1 v/v), acetone, absolute alcohol and distilled water, applying one after another with the same leaf powder. The extracted liquid was subjected to rotary evaporator in order to remove the chemicals. The resultant semisolid extract was kept in a deep freeze at -80 °C (REVCO model No, ULT 790-3-V32) for 12 h followed by freeze drying for 24 h at -60 °C. Then the extract was stored in an air tight container at 4 °C for further use. The dried precipitate were weighed and dissolved in suitable volume of distilled water to make different concentrations at mg/L levels.

2.5. Dose-dependent larvicidal bioassay

The larvicidal bioassay followed the WHO standard protocol [14] with suitable change. Each of the prepared concentrations of crude extract and solvent extracts was transferred into the sterile glass beaker (250 mL capacity). Twenty five early 3rd instar larvae of *Cx. quinquefasciatus* were introduced into different beaker containing appropriate concentrations. And 10 mg of larval food (dog biscuits: yeast extract=3:1) was added per beaker. Mortality rate were recorded after 24, 48 and 72 h of post-exposure. The data of mortality in 48 and 72 h were expressed by the addition of the mortality at 24 and 48 h, respectively for both crude and solvent extract experiments. The experiments were replicated four times on four different days.