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Adult emergence inhibition activity of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaf extracts against *Aedes aegypti* (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract
To determine adult emergence inhibition activity of *Cleistanthus collinus* crude leaf extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Adult emergence inhibition activity of crude aqueous leaf extracts of *Cleistanthus collinus* were assayed for their toxicity at concentrations of 500 and 1000 mg/L. Larval and pupal period lasted nine and three days in *Aedes aegypti* followed by ten and three days in *Anopheles stephensi* and *Culex quinquefasciatus* treated individuals. In the case of control it took eight and two days. Larval duration increased in treated individuals and total developmental period (larval and pupal development) took twelve, thirteen and thirteen days in *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* whereas in control it was ten days. The growth index was 2.5, 2.1 and 3.9 respectively. Adult emergence inhibition was noted in *Aedes aegypti* followed by *Anopheles stephensi* and *Culex quinquefasciatus* when larval and pupal development was arrested resulting in decreased pupal transformation. Larval and pupal periods were prolonged with appearance of larval–pupal and pupal–adult intermediates.

Keywords: *Cleistanthus collinus, leaf extracts, Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus, adult emergence inhibition activity.*

1. Introduction
Vector control is an essential requirement in control of epidemic diseases *viz*., malaria, filarial fever, Japanese encephalitis, dengue and chikungunya that are transmitted by mosquitoes. Excessive use of synthetic pesticides causes emergence of pesticide resistance and harmful effect on non-target organisms and environment. This has necessitated an urgent search for development of new and improved mosquito control methods that are economical and effective as well as safe for non-target organisms and the environment. Herbal insecticides of plant origin become a priority in this search [1]. The awareness of the harmful side effects on human and deleterious effect on the environment and development of resistance among vectors made concern over use of conventional synthetic insecticides for vector control and this paved way for the search for alternative control agents based on phytochemicals. Phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to reinstate synthetic insecticides under the integrated mosquito management programmes due to their notable mosquitocidal properties [2]. Many medicinally important plant extracts have been studied for their efficacy as mosquitocidal agent against different species of vector mosquitoes [3]. In recent years, use of conventional synthetic insecticides has been restricted due to their high cost, concern for environmental pollution, detrimental effect on human health, and other non-target populations, and increasing insecticide resistance on a global scale. There is therefore a clear requisite for the development of alternative mosquito control agents with different mode of action.
Cleistanthus collinus (Roxb.) is a toxic plant belonging to the family Euphorbiaceae and it grows in the dry forests of Southern and Central parts of India, Malaysia and Africa. It is commonly called as ‘garari’ in Hindi, ‘oduvan’ in Tamil, ‘vadise’ in Telugu and ‘nilapala’ in Malayalam [4]. Many parts of the plant were reported as toxic and the aqueous extract of the crushed laves of this plant are used as cattle and fish poison, abortifacient, suicidal and homicidal agents. The alcoholic extract of the leaves, roots and fruits of *Cleistanthus collinus*

are used to treat gastrointestinal disorders and it also possess anticancer activity. Further, the plant also possess insecticidal properties against the red flour beetle, *Tribolium castaneum* [4] and are used as insecticides in rice fields [5]. The leaf extracts of this plant exhibited, antifeedant and insect growth regulatory against the larvae of *Spodoptera litura* [6-8]. Further, the crude leaf extracts of *Cleistanthus collinus* was reported for larvicidal activity against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* [9]. Therefore, in the present study, the adult emergence inhibition activity of *Cleistanthus collinus* crude leaf extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* is studied.

2. Materials and Methods

2.1. Plant collection and extraction

Cleistanthus collinus leaves collected in and around Chennai, Tamil Nadu, India were brought to the laboratory; shade dried under room temperature and powdered using an electric blender. Dried and powdered leaves (1 Kg) was subjected to extraction using 3 L of distilled water for a period of 72 h to obtain crude aqueous extracts using rotary vacuum evaporator. The crude aqueous extracts thus obtained were lyophilized and a stock solution of 1,00,000 mg/L was prepared and refrigerated at 4 °C until testing for bioassays.

2.2. Test mosquitoes

All tests were carried out against laboratory reared vector mosquitoes *viz*., *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* free of exposure to insecticides. Cyclic generations of vector mosquitoes were maintained at 25-29 ºC and 80-90% R.H. in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on ten per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

2.3. Adult emergence inhibition bioassay

Standard protocol described by W.H.O. [10] was adopted for the study. The powdered plant leaves were put in cotton gauze sachets and immersed (for 6 h) in 250 mL beaker containing 200 mL water. Hundred early first instar larvae were exposed to the crude aqueous extract at concentrations of 500 and 1000 mg/L. A beaker containing distilled water (200 mL) only served as control. Dead larvae and pupae were removed and counted after 24 h. Observation on larval mortality, pupal mortality and adult emergence was recorded. The number of adults that failed to emerge from the pupae was counted in order to calculate the per cent inhibition. Growth index (GI) was calculated using the following formula [11].

$$GI = \frac{\text{Transformation of larva into adult (\%)}}{\text{Average developmental period in days}}$$

3. Results

Larval and pupal period lasted nine and three days in *Aedes aegypti* followed by ten and three days in *Anopheles stephensi* and *Culex quinquefasciatus* treated individuals. In the case of control it took eight and two days. Larval duration increased in treated individuals and total developmental period (larval and pupal development) took twelve, thirteen and thirteen days in *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* whereas in control it was ten days. The growth index was 2.5, 2.1 and 3.9, respectively and in control it was 9.1 in *Aedes aegypti*; 8.9 in *Anopheles stephensi* and 9.4 in *Culex quinquefasciatus*. The data also revealed gradual increase in pupal duration. Adult emergence against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* recorded at 500 and 1000 mg/L was 29.8 and 10.4; 26.4 and 4.8; 51.6 and 31.0, respectively. Among the three species of vector mosquito, *Aedes aegypti* was most susceptible followed by *Anopheles stephensi* and *Culex quinquefasciatus.* Student t-test analysis showed significant difference at $P < 0.001$ level on all three mosquito larval and pupal mortality treated with aqueous extracts (Table 1). Adult emergence inhibition suggested a general toxic effect of the extract, which was found to be dose dependent. The metamorphic abnormalities like larval inability to moult to next stage and larval pupal intermediates noticed were higher when compared to control group. Inability of adults to shed completely its exuvia, which remained attached to its appendages, was also noticed. The treated adult could not fly above normal level and rested for longer period on the water surface when compared to untreated adult mosquitoes. In this context of observation, exposure of larvae (all three vector mosquito species) to aqueous extract resulted in death at larval-pupal moult and pupal-adult eclosion suggesting inhibition of moulting process.

Table 1: Effect of *Cleistanthus collinus* aqueous leaf extracts on the growth and metamorphosis of vector mosquitoes

Vector mosquito species	Conc (mg/L)	Larval mortality (%)*	Total larval period in days	Pupal mortality (%)*	Total pupal period in days	Adult emergence (%)(a)	Total developmental period in days (b)	Growth index (a/b)
Aedes aegypti	**500**	62.0±3.24	9	8.2±0.84	3	29.8±3.77	12	2.5
	1000	73.4±2.41	9	16.2±1.30	3	10.4±2.88	12	0.9
	Control	8.2±0.84	8	1.2±0.84	2	90.6±0.89	10	9.1
Anopheles stephensi	**500**	61.0±2.92	10	12.6± 1.4	3	26.4± 3.29	13	2.1
	1000	78.8±2.59	10	16.4±1.14	3	4.8±3.27	13	0.4
	Control	9.2±1.48	8	1.4±0.89	2	89.4±2.30	10	8.9
Culex quinquefasciatus	**500**	41.8±2.95	10	6.6±1.14	3	51.6±3.85	13	3.9
	1000	56.4±3.29	10	12.6±2.41	3	31.0±2.65	13	2.3
	Control	4.4±1.82	8	1.8±0.84	2	93.8±2.28	10	9.4

*Significant at the level of $P < 0.001$ level

4. Discussion

Mosquitoes are continually developing resistance to the available insecticides and at present, there is no vaccine to prevent infections transmitted by mosquitoes. Vector control in the larval condition is the best available option as the larvae are confined to water bodies which are mainly man made and can be easily located [12]. Furthermore, for the protection of environment and other non-target organisms which share same habitat with the mosquito larvae, plant based insecticides are in demand for mosquito control as synthetic insecticides are non-biodegradable, toxic to environment and also responsible for resistance. Testing the plant crude extracts against mosquito can lead to identification of potential bioactive phytocompounds that can be used as larvicides to control mosquito. Botanical derivatives have drawn attention as potential insect control agents targeting the larval stages in the mosquito control programme in the last three decades [2, 13-16]. The efficacy of phytochemicals against mosquito larvae according to their chemical nature have described mosquitocidal potentiality of several plant derived secondary materials *viz*., alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans which has been reviewed [17]. The high rate of biodegradation exhibited by most phytochemicals makes them environmentally acceptable substitutes for synthetic chemicals as insect/mosquito control agents.

Many studies have drawn attention to the effects of plant extracts on adult eclosion [18, 19]. The benefit of elongation is that mosquito larvae numbers are reduced due to the longer period needed for a new generation to complete the life cycle [20]. Pushpalatha and Muthukrishnan [21] reported the leaf extracts of *Vitex negundo*, *Nerium oleander* and seed extract of *Syzygium jambolanum* at very low concentrations had effective larvicidal activity against *Culex quinquefasciatus* and *Anopheles stephensi* and also extended the duration of larval instars pupation. Murugan and Jeyabalan [22] reported that *Leucas aspera* has strong larvicidal, antiemergence activity against *Anopheles stephensi*. Sujatha *et al*. [23] observed *Acorus calamus* extract induced malformation to a greater extent in *Anopheles stephensi* larvae and to a lesser extent in *Culex quinquefasciatus* and *Aedes aegypti*. Daniel *et al*. [24] reported that there was a prolonged larval and pupal period on *Culex quinquefasciatus* when treated with the extracts of *Acalypha indica*. The petroleum ether extract of *Acorus calamus* and *Azadirachta indica* showed excellent insect growth regulatory effect against *Culex pipiens fatigans* [25]. Sharook *et al*. [26] found that acetone extracts from *Melia volkensii* and *Melia azedarach* seeds exhibited growth inhibitory activity against *Culex pipiens* larvae. Supavarn *et al*. [27] reported methanol extracts of whole plants of *Anethum graveolens* toxic to the fourth instar larvae of *Aedes aegypti* and high inhibition of pupal development. Mohtar *et al*. [28] reported methanol-aqueous extract of *Nerium indicum* leaves on different larval instars of *Aedes aegypti* and an elongation of the pre-imago period on the treated larvae. The biological activity of the plant extracts might be due to the saponins and alkaloids present in plants, since these compounds may jointly or independently contribute to produce larvicidal and adult emergence inhibition activity [29]. The crude extracts of *Chlorophytum borivilianum* tubers possess larvicidal and adult emergence inhibition activity against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. Sivagnaname and Kalyanasundaram [30] reported methanolic leaf extracts of *Atlantia monophylla* a as mosquitocide against immature stages of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* exhibiting insect regulating activity. Zebitz [31] reported azadirachtin as an anti-ecdysteroid, and it affects neuro endocrine control of ecdysteroids such that growth is inhibited and the larval developmental periods are prolonged.

Several studies have documented the efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito. But only a few have been commercially produced and extensively used in vector control programme [2]. The main reason for the failure in laboratory to field movements of bioactive toxic phytochemicals are poor characterization and inefficiency in determining the structure of active toxic ingredients responsible for mosquitocidal activity. The isolation of the active component could be useful as a biomarker in quality checking of each extract before moving to the field from the laboratory [32]. Targeting larvae, particularly in human-made habitats, can significantly reduce mosquito vector population particularly when applied in conjunction with indoor residual spraying (IRS) and other adulticidal measures. Botanical insecticides in combination with microbial biocontrol agents and insect growth regulators would be a good option for environmental friendly, toxicologically safe and community acceptable mosquito vector control programmes. Plant shows a vast range of phytochemicals which may be used in place of chemical pesticides due to their ecofriendly nature [1]. It may be concluded that natural products as extracts from parts of plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their insecticide toxicity [33]. Green synthesis of pesticides of biological origin may serve as suitable alternatives to synthetic or chemical insecticides in future as these are relatively safe, inexpensive, and are readily available in many areas of the world. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *Cleistanthus collinus* leaf extracts to control the immature stages of vector mosquitoes.

5. Acknowledgements

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