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Larvicidal efficacy of medicinal plant extracts against the vector mosquito *Aedes albopictus*

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Abstract
Mosquitoes are pestiferous vectors which are responsible for the transmission of various dreadful diseases, causing millions of deaths every year. Indiscriminate use of chemical insecticides has resulted in the development of resistance by these organisms, resulting in rebounding vectorial capacity. Moreover, such chemicals have given rise to serious environmental issues. This has led to the search for phytochemicals, which are having several advantages over the chemical insecticides in the control of vectors. The present endeavor is to assess the larvicidal ability of the aqueous extracts of ten medicinal plants against mosquito larvae *Aedes albopictus*, reared from eggs under laboratory conditions. Mortality percentages and LC_{50} were calculated. Among plants under experimentation, *Ocimum gratissimum*, *Phyllanthus emblica*, *Terminalia chebula*, *Aegle marmelos* and *Lantana camara* were the most effective.

Keywords: phytochemicals, *Aedes albopictus*, LC_{50}.

1. Introduction
Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of outrageous diseases like Malaria, Filariasis, Dengue, Japanese encephalitis, etc. causing millions of deaths every year [1, 2]. Eliminating the source of infection is an essential step in the control of mosquito-borne diseases [3]. During the past several decades, many synthetic organic insecticides have been developed and effectively used to eliminate mosquitoes. Unfortunately, the management of these disease vectors using synthetic insecticides has failed in part due to their efficiency in attaining physiological resistance. In addition, the application of such chemicals has resulted in long-term harmful effects on non-target organisms and other environmental components [4].
Most of the mosquito control programs target on the larval stage in breeding sites, as adulticides may only reduce the adult population temporarily [5, 6]. The conventional chemical methods employed for this purpose includes insecticides, Insect growth regulators (IGRs), Juvenile-hormone compounds etc. In recent times, chemicals derived from plants have been projected as weapons of future mosquito control programs as they are shown to be ecologically friendly. Moreover plant based bioproducts are mostly non-toxic to humans and other mammals and have a high degree of biodegradation [7, 8]. In view of an increasing interest in developing plant based insecticides as an alternative to chemical insecticides, the present study was undertaken to assess the larvicidal potential of the aqueous extracts of selected medicinal plants against mosquito larvae.

2. Materials and Methods
The present study has been carried out to assess the larvicidal activity of aqueous extracts of ten plants belonging to varied taxonomic groups (Table 1). The experiment was carried out in the following steps:

2.1 Plant collection and Processing
The selection of plants were carried out based on their local availability and reported medicinal properties. The materials were collected from the Botanical Garden of the University of Calicut (CUBG) and also from adjoining unpolluted areas. The materials were taken from healthy plants, free from dust, dirt and other impurities and were brought to the laboratory for subsequent processing.

2.2 Preparation of extracts
The washed and air dried plant materials were chopped properly and kept in clean trays. For the preparation of extracts, approximately 20 gms of plant material (leaf, flower, fruit, dry seed, and whole plant) were taken and ground in a homogenizer using distilled water. The extract was filtered and the filtrate was made up to 1000 ml with distilled water and retained as stock solution for further experimentation. Serial dilution of the stock solutions was carried out for assessing treatment efficiencies.

2.3 Selection, collection and culture of mosquito species
Vector species *Aedes albopictus* was selected for the present study. *Aedes albopictus* (Diptera: Culicidae) is an epidemiologically important vector involved in the transmission of many viral pathogens including yellow fever, dengue and chikungunya. As a vector species, the growth and multiplication of *Aedes albopictus* has proven to be very difficult to suppress or control due to their remarkable ability to adapt to various environments, their closeness with humans and their effective reproductive biology [9].

Mosquito larvae, collected from controlled breeding sites maintained with coconut shells kept at varying distances around households, were used in the present study. Collected larvae were pooled in the laboratory and subjected to species level identification using standard manual [10]. The screened larvae were reared to adults in the laboratory itself, to avoid mixing up of species. From these adults, F1 generation larvae were produced and used for the present study. This procedure also helped to maintain the uniform age of larval instar (Third instar). The larvae at all stages were reared in tap water (untreated) and fed with dog biscuits and yeast in the ratio 3:1. They were maintained at 28 ± 2 °C, 75–85% RH, under a 16 hr light and 8 hr dark cycle with 1 hr crepuscular period at the beginning and end of each light cycle.

2.4 Larvicidal bioassay
Bioassay for the larvicidal activity was carried out using WHO [11] procedure with minor modifications. Twenty larvae, each were introduced into treatment trays containing 250 ml of their natural growth medium (Tap water - untreated - added with dog biscuits and yeast in the ratio 3:1). To the treatment set, respective concentrations of the plant extracts (0.5, 1.0, 2.0, 4.0 and 8.0 ml) were added from the stock solution; maintaining a relative concentration of the plant extract as 10, 20, 40, 80 and 160 mg/ml respectively. A control was maintained, containing only larvae and natural growth medium. Mortality counts of larvae were monitored at regular intervals i.e. 6, 12, 24, 48, 72 and 96 Hours after Treatment. Larvae were considered dead if they settle and remain motionless in the bottom of the test beaker with no response to light or mechanical stimulus or not recovering life functions even after being transferred to their growth medium.

2.5 Statistical analysis
The concentration at which mortality observed (mg/ml) was corrected using Abbott's [12] formula. Statistical analysis of the experimental data was performed with MS Excel 2007 to find the Standard Deviation and LC_{50} using Probit Analysis [13].

3. Results and Discussion
The present study has been carried out to assess the lethality of the aqueous extracts of ten species of plants belonging to various families on mosquito larvae. Details of plants used for the present study and the mortality percentages of mosquito larvae noticed are depicted in Table 1. The effect of various plant extracts on mosquito larvae exposed to 96 hours, for confirming lethality as per WHO [11] standards are given in Table 2.

Table 1: List of plant species used for the preparation of aqueous extracts and results of their impact on *Aedes albopictus* larvae

Sl. No.	Name of the plant	Family	Part used	Condition at which larval mortality noticed	Mortality%
1	*Ocimum gratissimum*	Lamiaceae	Whole plant	8ml at 96hrs	100
2	*Adhatoda vasica*	Acanthaceae	Leaf	No Mortality	0
3	*Averrhoa bilimbi*	Oxalidaceae	Fruit	No Mortality	0
4	*Aegle marmelos*	Rutaceae	Leaf	8ml at 96 hrs	55
5	*Careya arborea*	Lecythidaceae	Fruit	No Mortality	0
6	*Commelina diffusa*	Commelinaceae	Leaf	No Mortality	0
7	*Lantana camara*	Verbenaceae	Flower	8ml at 96 hrs	90
8	*Phyllanthus emblica*	Phyllanthaceae	Dry seed	8ml at 96 hrs	95
9	*Scorparis dulis*	Plantaginaceae	Whole plant	No Mortality	0
10	*Terminalia chebula*	Combretaceae	Dry seed	8ml at 96 hrs	100

Table 2: Larvicidal activity of various plant extracts on mosquito larvae.

SI. No.	Plants	Concentration of the extract (ml in 250 ml of growth medium)						Mean±S.D
		Control	0.5	1	2	4	8	
1	*Ocimum grattissimum*	0	30	35	55	90	100	14±17.44
2	*Aegle marmelos*	0	0	0	0	10	55	21±28
3	*Lantana camara*	0	0	0	10	40	90	30±27.39
4	*Phyllanthus emblica*	0	40	70	70	95	95	67±35.72
5	*Terminalia chebula*	0	0	0	15	100	100	88±16.91

Significant differences were observed in the toxicity of the aqueous extracts of plants consisting of flower (1), leaf (3), Dry seed (2), whole plant (2) and fruit (2) against the third instar larvae of mosquitoes. Out of ten plants attempted, *Ocimum gratissimum* (Whole plant) and *Terminalia chebula* (Dry seed) were found to be capable of inducing 100% mortality against mosquito larvae at varying concentration and retention time. The plants like *Phyllanthus emblica*, (Dry seed) *Lantana camara* (Flower) and *Aegle marmelos* (Leaf), showed *95*, 90, and 55% larvicidal property at 8ml of the extract at 96 hours respectively. Extracts from the leaves of *Adhatoda vasica*, *Commelina diffusa* and fruit extract of *Averrhoa bilimbi*, *Careya arborea* and whole plant of *Scorparis dulis* were found to have no larvicidal efficiency (Table 1 and 2). The LC_{50} estimates for the promising plants were ranging from 1.08 to 9.12 mg/ml against the mosquito larvae (Figure 1).

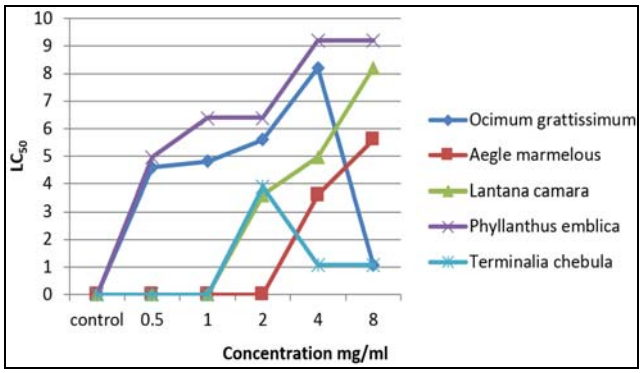

Fig 1: LC_{50} value of plant extract against mosquito larvae

Thus it is being noticed in the present study that the aqueous extracts prepared from *Ocimum gratissimum* (Lamiaceae), *Phyllanthus emblica* (Phyllanthaceae)**,** *Terminalia chebula* (Combretaceae)**,** *Aegle marmelos* (Rutaceae) and *Lantana camara* (Verbenaceae) has strong larvicidal activity against third instar larvae of *Aedes albopictus*. Sukumar *et al.* [14] has pointed out that the most promising mosquito control agents of botanical origin are from the families of Asteraceae, Lamiaceae, Meliaceae and Rutaceae. The present finding is a new addition to the list of plants being reported to have larvicidal properties.

Phytochemicals can thus be used as adequate alternatives to synthetic insecticides as they are relatively safe, inexpensive and are readily available throughout the world [15]. According to Bowers *et al.* [16] the biological activity of the plant extract is due to various compounds like alkaloids, terpenoids etc. synthesized within plants in varying proportions. These compounds, either independently or jointly contribute to larvicidal activity of mosquitoes. The current revise has opened up prospects for large scale extraction of active ingredients of plant origin for effective mosquito control. Also the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health [16].

In concise, the present study elucidated the larvicidal properties of plants belonging to five families in controlling vector mosquito *Aedes albopictus*. As stated earlier, the results reported in the present study open up the possibility of further investigations on evaluation, identification and isolation of the bioactive components of these plant extracts and its systematic effects on target mosquitoes, which would eventually facilitate the application of the extract as larvicidal, adult emergence inhibition and ovicidal agent in small-volume aquatic habitats or breeding sites of limited size in and around human dwellings.

4. Acknowledgement

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