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Plant diversity for mosquito control: A preliminary study

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Abstract
Mosquitoes adversely affect human health, as they act as vectors for a wide variety of dreadful diseases. Mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. In this context, phytochemicals have received much attention as potentially useful against mosquitoes. The present study has been outlined to assess the feasibility of using phytochemicals, derived from 36 species of plants, in the control of mosquito vectors belonging to the genera. The efficacy was determined through bioassay method as per WHO guidelines with slight modifications. Mortality percentages and LC_{50} were calculated after monitoring the treatment sets for a period of 96 hours. Of various plants studied, 29 showed 100% larvicidal activity against the third instar larvae of mosquitoes at varying concentrations of the extract. These plants have the potential to be used as effective larvicides, signifying an ecofriendly approach for the control of mosquito vectors.

Keywords: phytochemicals, Bio-assay, Mortality percentage, LC_{50}.

1. Introduction
Mosquitoes are the most important group of bloodsucking arthropods. They not only create nuisance to humans by biting, but also transmit serious diseases with many socioeconomic consequences. The situation has become worse over the last decade due to global climatic changes. This, in addition to other factors has favored them to adapt to a wide range of habitats and thereby to increase its population in many parts of the world [8]. Several mosquito species belonging to the genera *Anopheles, Culex* and *Aedes* are acting as vectors for many pathogenic organisms causing diseases like Malaria, Filariasis, Japanese Encephalitis, Dengue fever, Yellow fever etc. These diseases spread globally, causing high levels of human mortality and thereby acting as factors impedimenting the economic development of most of the developing countries across the world [3]. Various pesticides and chemical formulations have been employed in an effort to control or eradicate mosquito populations. Even though they are highly efficacious against the target species, these pesticides are facing threats due to the development of resistance to chemical insecticides by the mosquitoes, resulting in rebounding vectorial capacity [12]. The long-term stability of many of these pesticides and their tendency to bio accumulates in non-target organisms have fostered many environmental and human health concerns. Plant secondary metabolites are considered to be a potential alternative approach against different species of mosquitoes and their various immature stages due to their richness in bioactive compounds, easy availability, environmental safety etc. [10]. As part of continued search for plant extracts with larvicidal properties, the activity of crude aqueous extracts of 36 plants were worked out in the present study. The findings of the studies in this direction will be useful in promoting research aiming at the development of new agents for mosquito control from indigenous plant sources.

2. Materials and Methods
The present study has been carried out to assess the larvicidal activity of aqueous extracts of 36 plants belonging to varied taxonomic groups. The experimentation has been carried out in the following steps:

2.1 Plant collection and Processing
The selection of plants was carried out based on their local availability and reported medicinal properties. The materials were collected 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 plant materials were chopped properly and kept in clean trays. For the preparation of extracts, approx. 20 gms of plant material was 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 a stock solution for further experimentation. Serial dilutions of the stock solutions were prepared for assessing treatment efficiencies.

2.3 Larval Screening and Experimentation
Mosquito larvae, collected from controlled breeding sites maintained with coconut shells kept at varying distances round households were used in the present study. Collected larvae were pooled in the laboratory and subjected to species level identification using standard manual [2]. 20 larvae, each were introduced into treatment trays containing 250 ml of their natural growth media. The efficacy was determined through bioassay method [13]. To the treatment set, varying concentrations of the plant extracts (ie. 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 also maintained for the treatment set. 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 settled and remained motionless at the bottom of the treatment tray with no response to light or mechanical stimulus or not recovering life functions even after being transferred to a control water solution.

2.4 Statistical analysis
The mortality observed (mg/ml) was corrected using Abbott's [1] formula during the observation of the larvicidal potentiality of the plant extracts. Statistical analysis of the experimental data was performed with MS Excel 2007 to find the Standard deviation and LC_{50} using probit analysis [5].

3. Results
The present study has been carried out to assess the lethal properties of 36 species of plants belonging to 27 families, in the control of mosquito larvae. Details of plants used for the present study and conditions at which highest mortality has been 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 [13] standards is given in Table 2.

Table 1: List of plant species used for the preparation of aqueous extracts and their impact on Mosquito larvae.

Sl. No.	Name of plant	Family	Part used	Mosquito genera	Condition at which larval mortality noticed	Mortality %
1	*Adenocalymma alliaceum*	Bignoniaceae	Leaf	*Aedes, Mansonia, Culex*	1ml at 72 hrs	100
2	*Allium sativum*	Amaryllidaceae	Bulb	*Aedes, Culex*	1ml at 12 hrs	100
3	*Alpinia galanga*	Zingiberaceae	Leaf	*Aedes*	1ml at 72 hrs	100
4	*Artocarpus heterophyllus*	Moraceae	Leaf	*Aedes*	8 ml at 96 hrs	100
5	*Azadirachta indica*	Meliaceae	Leaf	*Aedes, Mansonia, Culex*	1 ml at 24 hrs	100
6	*Bacopa monnieri*	Plantaginaceae	Leaf	*Aedes, Culex*	4 ml at 96 hrs	100
7	*Calotropis gigantea*	Apocynaceae	Leaf	*Aedes*	1 ml at 96 hrs	100
8	*Capsicum annuum*	Solanaceae	Herb	*Aedes*	8 ml at 96 hrs	95
9	*Carica papaya*	Caricaceae	Leaf	*Aedes*	4 ml at 96 hrs	100
10	*Chromolaena odorata*	Asteraceae	Leaf	*Aedes, Culex*	4 ml at 72 hrs	100
11	*Chrysanthemum indicum*	Asteraceae	Leaf	*Aedes*	2 ml at 72 hrs	100
12	*Citrus sinensis*	Rutaceae	Fruit peel	*Aedes*	0. 5 ml at 6 hrs	100
13	*Cleome viscosa*	Capparaceae	Herb	*Aedes*	8 ml at 96 hrs	85
14	*Clerodendron infortunatum*	Verbenaceae	Aerial part	*Aedes*	8 ml at 96 hrs	100
15	*Cymbopogon citratus*	Poaceae	Leaf	*Aedes*	8 ml at 96 hrs	100
16	*Gliricidia maculata*	Fabaceae	Leaf	*Aedes*	8 ml at 72 hrs	100
17	*Glycosmis pentaphylla*	Rutaceae	Leaf	*Aedes*	8 ml at 96 hrs	100
18	*Mangifera indica*	Anacardiaceae	Leaf	*Aedes*	8 ml at 96 hrs	100
19	*Mentha spicata*	Lamiaceae	Leaf	*Aedes*	4 ml at 72 hrs	100
20	*Millettia pinnata*	Fabaceae	Leaf	*Aedes*	8 ml at 96 hrs	65
21	*Mimosa pudica*	Leguminosae	Herb	*Aedes*	8 ml at 48 hrs	100
22	*Momordica charantia*	Cucurbitaceae	Unripe fruits	*Aedes, Culex*	0.5 ml at 12 hrs	100
23	*Musa paradisiaca*	Musaceae	Peduncle	*Aedes*	0.5 ml at 24 hrs	100
24	*Ochroma grandiflorum*	Malvaceae	Flower	*Armigeres*	0.5 ml at 12 hrs	100
25	*Ocimum gratissimum*	Lamiaceae	Leaf	*Aedes, Mansonia*	8 ml at 48 hrs	100
26	*Opuntia dillenii*	Cactaceae	Cladodes	*Aedes, Mansonia*	8 ml at 48 hrs	85
27	*Phyllanthus emblica*	Phyllanthaceae	Dry Seed	*Aedes*	8 ml at 96 hrs	95
28	*Physalis minima*	Solanaceae	Herb	*Aedes, Armigeres*	8 ml at 72 hrs	100
29	*Polyalthia longifolia*	Annonaceae	Leaf	*Aedes*	0.5 ml at 48 hrs	100
30	*Ricinus communis,*	Euphorbiaceae	Seed	*Aedes, Armigeres*	0.5 ml at 6 hrs	100
31	*Saritaea magnifica*	Bignoniaceae	Leaf	*Aedes*	0.5 ml at 24 hrs	100
32	*Sida cordifolia*	Malvaceae	Herb	*Aedes*	4 ml at 72 hrs	100
33	*Trigonella foenum*	Fabaceae	Dry Seed	*Aedes*	8 ml at 96 hrs	90
34	*Vernonia cinerea*	Asteraceae	Flower	*Aedes*	8 ml at 96 hrs	35
35	*Vitex negundo*	Verbenaceae	Leaf	*Aedes, Armigeres, Culex, Mansonia*	4 ml at 72 hrs	100
36	*Ziziphus jujuba*	Rhamnaceae	Dry Seed	*Aedes*	8 ml 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	*Adenocalymma alliaceum*	0	100	100	100	100	100	100±0
2	*Allium sativum*	0	100	100	100	100	100	100±0
3	*Alpinia galanga*	0	100	100	100	100	100	100±0
4	*Artocarpus heterophyllus*	0	0	0	0	35	100	27±38.94
5	*Azadirachta indica*	0	100	100	100	100	100	100±0
6	*Bacopa monnieri*	0	20	15	90	100	100	65±19.47
7	*Calotropis gigantea*	0	95	100	100	100	100	99±17.96
8	*Capsicum annuum*	0	0	10	25	65	85	37±16.63
9	*Carica papaya*	0	0	0	15	100	100	43±15.50
10	*Chromolaena odorata*	0	0	20	80	80	100	56±14.54
11	*Chrysanthemum indicum*	0	85	80	100	100	100	93±13.73
12	*Citrus sinensis*	0	100	100	100	100	100	100±0
13	*Cleome viscosa*	0	0	0	15	50	90	31±12.96
14	*Clerodendron infortunatum*	0	0	0	35	75	100	42±12.41
15	*Cymbopogon citrates*	0	0	0	5	20	100	25±11.93
16	*Gliricidia maculate*	0	0	0	25	55	100	36±11.49
17	*Glycosmis pentaphylla*	0	0	0	40	90	100	46±11.10
18	*Mangifera indica*	0	0	0	0	55	100	31±10.75
19	*Mentha spicata*	0		15	20	100	100	47±10.44
20	*Millettia pinnata*	0	5	5	15	40	50	23±10.15
21	*Mimosa pudica*	0	65	35	85	55	100	68±9.88
22	*Momordica charantia*	0	100	100	100	100	100	100±0
23	*Musa paradisiaca*	0	100	100	100	100	100	100±0
24	*Ochroma grandiflorum*	0	100	100	100	100	100	100±0
25	*Ocimum gratissimum*	0	30	35	55	90	100	62±9.80
26	*Opuntia dillenii*	0	0	0	0	25	85	22±9.59
27	*Phyllanthus emblica*	0	0	0	0	60	95	31±9.40
28	*Physalis minima*	0	0	10	25	65	85	37±9.22
29	*Polyalthia longifolia*	0	100	100	100	100	100	100±0
30	*Ricinus communis,*	0	100	100	100	100	100	100±0
31	*Saritaea magnifica*	0	100	100	100	100	100	100±0
32	*Sida cordifolia*	0	20	40	80	100	100	68±9.10
33	*Trigonella foenum*	0	15	50	65	80	90	60±8.95
34	*Vernonia cinerea*	0	0	40	50	70	85	49±8.81
35	*Vitex negundo*	0	60	60	55	70	100	69±8.67
36	*Ziziphus jujuba*	0	0	0	10	50	100	32±8.54

Systematic analysis of mosquito larvae using standard manual "The Fauna of British India" [2] revealed that they are falling in four genera, *Aedes*, *Culex*, *Armigeres* and *Mansonia*. Out of 36 plants attempted, 29 were found to be capable of inducing 100% mortality against mosquito larvae at varying concentration and retention time. Of the 29 plants, extracts from *Citrus sinensis*, *Ricinus communis*, *Momordica charantia*, *Ochroma grandiflorum*, *Musa paradisiaca*, *Saritaea magnifica*, *Polyalthia longifolia*, showed 100% larvicidal property at 0.5 mg/ml concentration and varied retention time (Fig. 1). Extracts from *Azadirachta indica*, *Allium sativum*, *Adenocalymma alliaceum*, *Alpinia galanga*, *and Calotropis gigantea* showed 100% larvicidal property at 1mg/ml concentration and varied retention time (Fig. 2). Extracts from *Carica papaya*, *Chromolaena odorata*, *Mentha spicata*, *Sida cordifolia*, *Vitex negundo*, *Bacopa monnieri* showed 100% larvicidal property at 4 mg/ml concentration at 72 and 96 hrs (Fig. 3). The other plant extracts showed moderate larvicidal activity compared to others. Among these plants *Chrysanthemum indicum* showed 100% mortality at 2 mg/ml concentration at 72 hours (Fig. 4, 5).

The LC_{50} estimates for the promising plants were 10.8 mg/ml respectively.

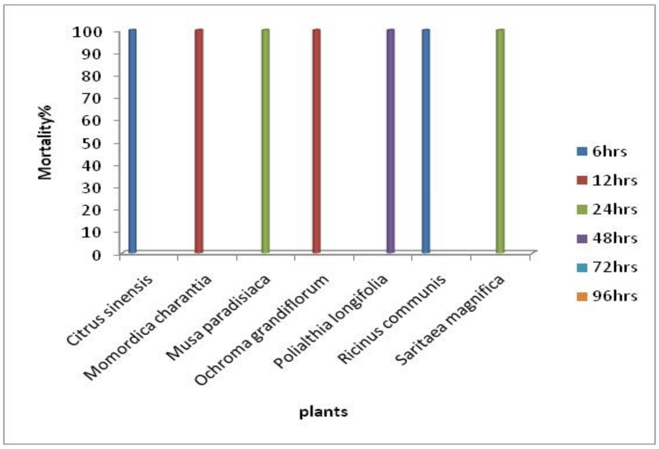

Fig 1: Plants showing mortality% at 0.5mg/ml concentration

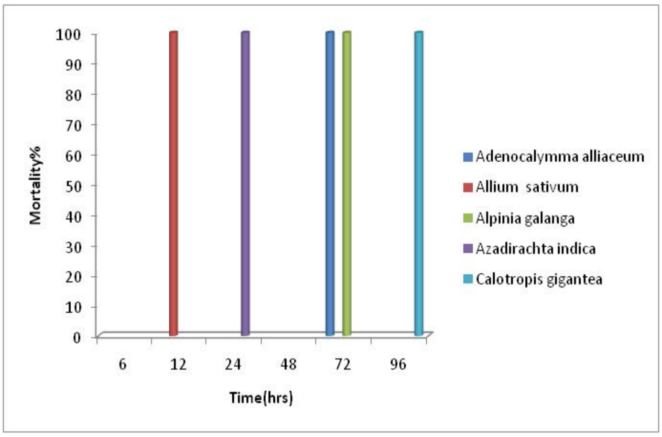

Fig 2: Plants showing mortality% at 1 mg/ml concentrations

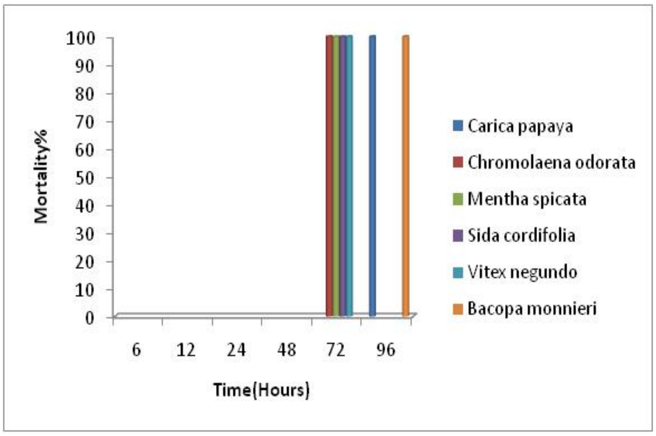

Fig 3: Plants showing mortality% at 4 mg/ml concentration

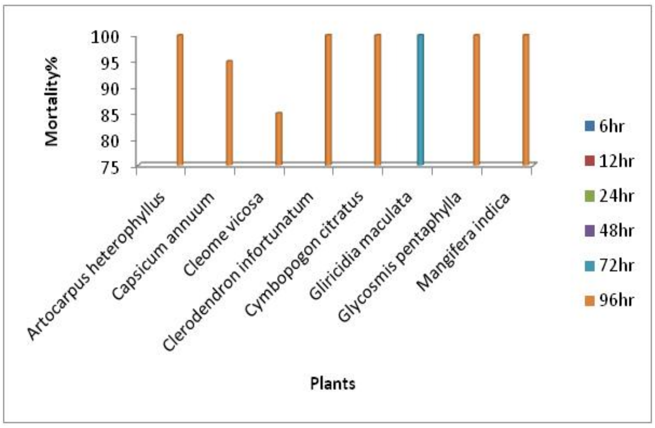

Fig 4: Plants showing mortality% at 8 mg/ml concentration

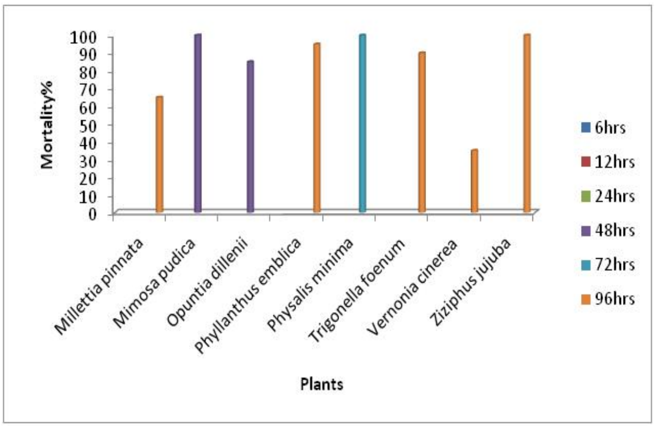

Fig 5: Plants showing mortality% at 8 mg/ml concentration

4. Discussion

In the present study, it has been noted that aqueous extracts of *Citrus sinensis* (Fruit peel), *Ricinus communis* (Seed), *Momordica charantia* (Unripe fruit), *Ochroma grandiflorum* (Flower), *Musa paradisiacal* (peduncle), *Saritaea magnifica* (leaf), and *Polyalthia longifolia* (Leaf) can be utilized for controlling mosquito larvae. Samuel Tennyson [11] pointed out that a considerable number of plant derivatives have shown to be effective against mosquitoes with a safe manner. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices. Kamaraj *et al.* [7] find that leaf and bark extract of *Annona squamosa*, *Chrysanthemum indicum* and *Tridax procumbens* can be developed as ecofriendly larvicides. The present finding is a new addition to the list of plants having larvicidal property, being reported by EI Kamali [4], Narayan G and Narayanapillai KG [9], Kalyanasundaram and Das [6]. The present study also gains significance as the plant / plant parts which are found to be effective are either perennially available in large quantities or available with ease and little cost. The study has opened up prospects for large scale extraction of active ingredients of plant origin for effective mosquito control. Also, the present study gains more importance as nontarget organisms in the ecosystems have been little affected by the application of these plant extracts.

In concise the primary objective of this work was to find out an effective means for controlling mosquito larvae using aqueous extracts of plant origin. The present study revealed that out of 36 plant species attempted, *Citrus sinensis* (Fruit peel), *Ricinus communis* (Seed) *Momordica charantia* (Unripe fruit), *Ochroma grandiflorum* (Flower), *Musa paradisiacal* (peduncle), *Saritaea magnifica* (Leaf) and *Polyalthia longifolia* (Leaf) have the potential to be included in the formulations of new and safe control products against mosquito vectors. As these plant species are distributed throughout the country, it can help minimize the dependence on expensive synthetic pesticides, generate local employment and also to stimulate local efforts to enhance public health. Further studies on the larvicidal mode of action, their effects on non-target organisms and formulations for improving their insecticidal potency are to be carried out for their standardization.

5. Acknowledgement

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