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Bioefficacy of botanical insecticides against the dengue and chikungunya vector *Aedes aegypti* (L.) (Diptera: Culicidae)

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ABSTRACT

Objective: To determine the bioefficacy of plant extracts *viz.*, whole plants of *Sphaeranthus indicus* (Asteraceae) and *Citrullus colocynthis* (Cucurbitaceae), leaves of *Abutilon indicum* (Malvaceae), *Cleistanthus collinus* (Euphorbiaceae), *Leucas aspera* (Lamiaceae) and *Murraya koenigii* (Rutaceae), and aerial parts of *Hyptis suaveolens* (Lamiaceae) against the dengue and chikungunya vector *Aedes aegypti*. **Methods:** The larvicidal activity was determined against the early third instar larvae at concentrations of 250, 500, 750 and 1000 ppm. Larval mortality was assessed after 24 h. **Results**: The ethyl acetate extract of *Sphaeranthus indicus* (201.11ppm) and hexane extract of *Abutilon indicum* (261.31ppm) was found to be effective. **Conclusions**: Further in-depth investigations on the crude extract/phytotoxic compounds of *Sphaeranthus indicus* are needed to elucidate the larvicidal activity against a wide range of all stages of mosquito species and also the active ingredients of the extract responsible for larvicidal activity in *Aedes aegypti* should be identified, and small scale field trials are needed for usage of this plant as a mosquitocidal agent.

1. Introduction

Mosquitoes (Diptera: Culicidae) represent a significant threat to human health because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide. Several species belonging to genera *Aedes*, *Anopheles* and *Culex* are vectors for the pathogens of various diseases like Dengue fever, Dengue haemorrhagic fever, Malaria, Japanese encepahalitis and Filariasis [1]. *Aedes aegypti*, the vector of dengue is widely distributed in the tropical and subtropical zones and dengue haemorraghic fever is endemic to South East Asia, the Pacific Islands, Africa and America [2]. One of the successful way of reducing mosquito densities is by attacking the larval breeding sites [3]. Plant products have been used traditionally by human communities and application of easily degradable plant compounds is considered to be one of the safest methods of control of insect pests and vectors. Plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control [4]. Development of resistance by pests and vectors against the botanicals has not been reported and botanical insecticides are generally pest specific, readily biodegradable, target specificity, lower bioaccumulation and lack toxicity to higher animals [5, 6]. Therefore, the present study has been carried out to evaluate the larvicidal activity of whole plants of *Sphaeranthus indicus* (Asteraceae) and *Citrullus colocynthis* (Cucurbitaceae), leaves of *Abutilon indicum* (Malvaceae), *Cleistanthus collinus* (Euphorbiaceae), *Leucas aspera* (Lamiaceae) and *Murraya koenigii* (Rutaceae), and aerial parts of *Hyptis suaveolens* (Lamiaceae) extracts against the dengue and chikungunya vector, *Aedes aegypti* (L.).

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2.MATERIALS AND METHODS

2.1 Plant extracts

Seven plant extracts *viz.*, whole plants of *Sphaeranthus indicus* (Asteraceae) and *Citrullus colocynthis* (Cucurbitaceae), leaves of *Abutilon indicum* (Malvaceae), *Cleistanthus collinus* (Euphorbiaceae), *Leucas aspera* (Lamiaceae), and *Murraya koenigii* (Rutaceae) and aerial parts of *Hyptis suaveolens* (Lamiaceae) collected in and around Chennai, Tamilnadu, India were brought to the laboratory, shade dried under room temperature and powdered using an electric blender. Dried and powdered plant parts of each plant (1 kg) was subjected to sequential extraction using 3 L of hexane, diethyl ether, dichloromethane and ethyl acetate for a period of 72 hours each to obtain the crude extracts using rotary vacuum evaporator. The hexane, diethyl ether, dichloromethane and ethyl acetate crude extracts thus obtained were lyophilized and a stock solution of 1,00,000 ppm prepared from each crude extract by adding adequate volume of acetone was refrigerated at 4 ℃ until testing for bioassays.

2.2 Test mosquitoes

Tests were carried out against laboratory reared *Aedes aegypti* mosquitoes free of exposure to insecticides and pathogens. Cyclic generation of *Aedes aegypti* were maintained at 25-29 °C and 80-90 per cent 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 Larvicidal bioassay

Bioassay for the larvicidal activity was carried out using WHO [7] procedure with slight modifications. From the stock solution, concentrations of 250, 500, 750 and 1000 ppm was prepared. Twenty five early third instar larvae were introduced into a 250 ml glass beaker containing 200 ml of water with each concentration. A total of three trials were carried out with five replicates per trial. Control was prepared by the addition of acetone to water. Mortality was recorded after 24 hours and the control mortality was corrected using Abbott's [8] formula,

$$\text{Per cent mortality} = \frac{\%\ \text{Mortality in treated} - \%\ \text{Mortality in control}}{100 - \%\ \text{Mortality in control}} \times 100$$

2.4 Statistical analysis

SPSS [9] was used for determination of LC_{50} and LC_{90} values. Data from mortality and effect of concentrations were subjected to analysis of variance. Difference between the treatments was determined by Tukey's test ($P < 0.05$).

3. RESULTS

The effect of various botanical extracts on the mortality

Table 1

Probit analysis of larvicidal bioefficacy of plant extracts against *Aedes aegypti*

Extracts	LC50(ppm)	LC90(ppm)	Chi-squarevalue	Regression value
Sphaeranthus indicus (Asteraceae)				
Hexane	966.03	4229.94	1.33*	1.69
Diethyl ether	869.37	4082.91	5.20*	1.91
Dichloromethane	1559.56	8958.67	0.01*	1.69
Ethyl acetate	201.11	865.83	17.86	2.02
Citrullus colocynthis (Cucurbitaceae)				
Hexane	1087.64	3752.82	1.90*	2.38
Diethyl ether	1022.36	5122.13	2.24*	1.83
Dichloromethane	515.69	1725.59	19.01	2.44
Ethyl acetate	1212.96	4682.91	0.28*	1.18
Abutilon indicum (Malvaceae)				
Hexane	261.31	1196.20	31.35	1.94
Diethyl ether	1442.34	5691.63	0.99*	2.15
Dichloromethane	1434.59	5932.17	0.59*	2.08
Ethyl acetate	898.87	2580.90	1.57*	2.80
Cleistanthus collinus (Euphorbiaceae)				
Hexane	1291.21	5070.99	0.03*	2.16
Diethyl ether	837.36	3450.87	4.98*	2.08
Dichloromethane	755.26	2831.15	3.71*	2.23
Ethyl acetate	560.41	2669.86	7.10	1.89
Leucas aspera (Lamiaceae)				
Hexane	1359.25	6751.97	0.05*	1.84
Diethyl ether	927.62	4308.47	0.21*	1.23
Dichloromethane	844.63	4251.98	3.53*	1.83
Ethyl acetate	483.21	3195.91	7.19	1.56
Murraya koenigii (Rutaceae)				
Hexane	856.70	3771.08	4.37*	1.99
Diethyl ether	511.12	1882.24	0.92*	1.75
Dichloromethane	1452.54	3719.80	3.42*	3.14
Ethyl acetate	1421.09	4927.15	6.39*	2.37
Hyptis suaveolens (Lamiaceae)				
Hexane	543.66	3546.69	7.49	1.57
Diethyl ether	1443.53	8362.41	2.05*	1.68
Dichloromethane	1292.36	6016.58	0.23*	1.92
Ethyl acetate	853.04	3549.90	2.52*	2.07

*Significant at $P < 0.05$ level

of *Aedes aegypti* larvae are presented in Table 1. The ethyl acetate extract of *Sphaeranthus indicus* whole plant was found to be effective with a LC_{50} value of 201.11 ppm against the larvae of *Aedes aegypti* followed by hexane leaf extract of *Abutilon indicum* with 261.31 ppm.

4. DISCUSSION

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides. However due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides, better alternative means are sought. A considerable number of plant derivatives have been screened effective against mosquitoes [10, 11]. Though several plant species from different families have been reported for mosquitocidal activity, only few botanicals have moved from laboratory to field use which might be due to the presence of phytochemicals when compared to synthetic insecticides. Larvae from the three medically important mosquito genera *Aedes, Anopheles* and *Culex* are all susceptible to a greater or lesser extent to some phytochemicals [11]. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices. Plants belonging to different families have been extensively screened/studied for their larvicidal activity ever since the discovery of the larvicidal potential of the extract of *Chrysanthemum cinnerariaefolium* [12]. Crude extracts of many plants showed larvicidal activity against mosquitoes [13]. Ethyl acetate leaf extract of *Eclipta prostrata* (LC_{50} value of 119.89ppm) was found to be effective against *Culex tritaeniorhynchus* [14], *Achilea millefolium* methanolic stem extract (LC_{50} value of 120.0ppm) against *Culex quinquefasciatus* [15], petroleum ether extract of *Centratherum anthelminticum* fruits (LC_{50} value of 162.60ppm) against *Anopheles stephensi* [16], *Tanacetum vulgare* methanolic flower extract (LC_{50} value of 178.0ppm), methanolic stem extract of *Otanthus maritimus* (LC_{50} value of 195.0ppm) [17] and ethyl acetate leaf extract of Strychnos nuxvomica against Culex quinquefascaitus [18] and petroleum ether leaf extracts of *Artemisia nilagirica* against *Culex quinquefasciatus, Galinsoga quadriradiata* against *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*, *Tagetes erectes* against *Anopheles stephensi* and *Aedes aegypti* [19] were comparable with the results of the present study. Therefore, further in-depth investigations on the crude extract/phytotoxic compounds of *Sphaeranthus indicus* are needed to elucidate the larvicidal activity against a wide range of all stages of mosquito species and also the active ingredient of the extract responsible for larvicidal activity in *Aedes aegypti* should be identified, and small scale field trials are needed for usage of this plant as a mosquitocidal agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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