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Review Article

Larvicidal activity of phytoextracts against dengue fever vector, *Aedes aegypti* - A review

K V Lakshmi[1,2], A V Sudhikumar[1], E M Aneesh[2*]

[1] Department of Zoology, Christ College, Irinjalakuda, Kerala, India
[2] Communicable Disease Research Laboratory, Department of Zoology, St. Joseph's College, Irinjalakuda, Kerala, India

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****Correspondence***
E. M. Aneesh
✉ aneeshembalil@gmail.com

Abstract

Since *Aedes aegypti* is considered as the major vector of dengue fever, development of strategies to accomplish improved vector control without much interference in the environment composition are more common. As phytochemicals are now in the run for achieving this goal, this review is a humble attempt to recognize the plant species and their larvicidal efficacy with their inhibitory action on the life cycle of the species of interest, that has been documented through various studies conducted till date. Here we also discuss the synergistic impact of a number of phytoextracts which will provide more efficient control measures for mosquito vectors. All these studies are an exploration for a risk-free vector control tactic to replace the current chemical insecticide application for the betterment of our nature.

Keywords

Aedes aegypti; phytoextracts; larvicidal activity; susceptibility

Citation

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Introduction

Mosquitoes are considered as the principle vectors of several diseases affecting humans and animals which include dengue, filariasis, chickungunya, Japanese encephalitis, malaria, etc, which result in thousands of deaths every year. Among these, dengue fever is measured as one of the noxious diseases due to its high mortality rate and increasing pervasiveness (1). The rate of recurrence of dengue has grown significantly around the globe in current times. According to recent reports, it is estimated that almost three ninety million people across the world are infected per year of which ninety million are in severe state (2,3). Most of the outbreaks of this disease remain unpredictable and are spreading to new areas, which result in an irrepressible increase in the occurrence of cases. Based on the data collected by National Vector Borne Disease Control Programme in India, dengue fever was first accounted in 1956 from Vellore

District in Tamil Nadu and the first dengue haemorrhagic fever outburst was informed from the eastern coast in 1963 (4). Transmission of dengue fever virus to humans is through mosquitoes of the genus *Aedes*, mainly by *Aedes aegypti,* hence known as the most important dengue vector (5).

Research has proved that the most triumphant method for the diminution of such vector borne diseases is the successful control of their vector organisms. Different methods were pursued to implement this purpose from ancient periods including physical, chemical, mechanical, and biological methods, of which chemical method has been demonstrated to be the most effective.

Phytochemicals were used as a crucial mosquito vector managment tactic since 1920's and they were gradually replaced by the synthetic chemicals after the induction of DDT. In an earlier study, it was reported that the plant alkaloids resembling nicotine, anabasine, methyl anabasine and lupinine extracted from a weed *Anabasis aphylla,* had highly efficient larvicidal activity (6). Literature review in this sector suggested that more than 1200 plants were listed for their potential insecticidal value in the early 90's (7). They almost disappeared from the field after the discovery of the synthetic insecticides such as organochlorides, organophosphates, carbamates, DDT and pyrethroids (8).

Insecticides that contain compounds of high mortality rates have been used worldwide for the management of insect pests including mosquitoes. Even though these insecticides supported intrusions have efficiently controlled mosquito populations for several years, dependence on these limited active ingredients and their profound use has caused progression of resistance against these compounds (9). Studies on resistance development have suggested that, *Aedes aegypti* has triggered resistance to all insecticides including carbamates, organochlorides, organophosphates and pyrethroids (10). Resistance development and more prominently the environmental catastrophe have compelled the researchers to revert their corridors once again towards phytochemicals for achieving successful vector control devoid of such tribulations.

Phytoextracts

Botanicals are principally secondary metabolites that operate as a means of defence mechanism of the plants to resist continuous selection pressure from herbivore predators and other environmental factors and some of them exhibit natural insecticidal activity as well (11). An assessment of earlier studies gives comprehensive evidence for the utilization of plant products as insecticides against mosquito populations. Extraction and evaluation of phytochemicals from 150 plants were carried out and their larvicidal effects were listed by Hartzeall and Wilcoxon in 1941 (12). In a previous review performed on insecticides derived from plants, covering a period of 1941 to 1953, it was reported that several phytochemicals were in use against mosquitoes for their control (13). Developing resistance against chemical insecticides made this harmless method more popular amongst researchers and common people alike. Present studies are focusing on these phytoextract based vector control strategies and also developing a newer and more effective method called synergy where a combination of more than one compound either of plant products or a combination of plant product and chemical insecticides are used for accomplishing the objective.

Same phytochemicals exhibit different susceptibility status to different mosquito species. Among the key vector genera like *Aedes* and *Culex*, *Aedes* mosquitoes are less susceptible to insecticides and botanicals (11). Since resistance development and environmental peril have been revealed for conventional insecticides, a range of studies on plant products as the mosquito vector control measures are being carried out by the researchers. Wide array of these natural compounds have been tested for this purpose including thousands of plant species. In this review, the plant extracts that have accounted for larvicidal efficacy against *Aedes* mosquitoes and their lethal dose are listed (Table 1). Various plants having larvicidal efficacy were subjected to further studies for identification of the chemical compounds which specifically possess larvicidal activity. Many compounds of interest have been recognized and isolated from various plant species of which important ones are listed in Table 2.

To compete with the increasing resistance development in mosquito species, a newer and faster method of vector control has been formulated in which synergistic action of more than one insecticide is exploited in combination with one another to enhance their potency for mosquito control. Studies have proven that the synergistic combination of biological and chemical insecticides yield a promising alternative resolution in vector management (14). At present, many synergistic combinations are under experimentation which includes the synergy of chemical and biological insecticides and mixtures of different phytoextracts alone.

When the leaf extracts of *Solidago canadensis* and *Eugenia jambolana* were combined with deltamethrin for the synergistic activity had a synergistic factor of 4.09 and 1.80. They were observed to give more efficient control than the phytoextracts alone (15). Synergistic efficacy of *Vitex negundo*, *Clerodendrum inerme* and *Gliricidia sepium* with *Pongamia glabra* against *Aedes aegypti* provided a synergistic factor of 1.90, 1.50 and 1.72 respectively and revealed that synergy of phytoextracts also offer proficient vector control in which only the combinations of phytoextracts were entailed (16).

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Table 1: Efficacy of botanical extracts in controlling/reducing the populations of *Aedes aegypti*, a dengue fever vector (LC: Lethal Concentration, ppm: parts per million, mg/L: milligram per liter)

Plant species	Plant families	Plant part	Solvent	Lethal concentrations or biological activity	References
Caulerpa scalpelliformis	Caulerpaceae	Whole	Acetone	LC: 53.7mg/L	(21)
Cannabis sativa	Cannabaceae	Leaf	Ethanol	LC: 5000mg/L	(22)
Codiaeum variegatum	Euphorbiaceae	Leaf	Water	LC: 37,600 mg/L	(23)
Azadirachta indica	Meliaceae	Leaf	Water	LC: 4800 mg/L	(23)
Annona squamosa	Annonaceae	Leaf	Water	LC: 2400 mg/L	(23)
Azadirachita indica	Meliaceae	Seed	Crushed seeds	LC: 100 (59) mg/L	(24)
Angelico glauca	Apiaceae	Seeds	Commercial oils	LC: 52–74 mg/L	(25)
Calophyllum inophyllum	Calophyllaceae	Leaf	Ethyl-acetate fraction	LC: 35.49 mg/L	(26)
Calophyllum inophyllum	Calophyllaceae	Seed	Ethyl-acetate fraction	LC: 8.2 mg/L	(26)
Alnus glutinosa	Betulaceae	Old litter	Polyphenols	LC: 200–400 mg/L	(17)
Abuta grandifolia	Menispermaceae	Fruit	Dichloro methane	LC: 2.6 mg/L	(27)
Feronia limonia	Rutaceae	Leaf	Acetone	LC_{50}:57.23 LC_{90}:146.21 mg/L	(28)
Alnus glutinosa	Betulaceae	Different aged litter		LC_{50}:0.382 g/L	(18)
Cassia obtusifolia	Fabaceae	Seed	Methanol	LC: 40 (51) mg/L	(29)
Cassia tora	Caesalpinaceae	Seed	Methanol	LC:20 (59) mg/L	(29)
Callitris glaucophylla	Cupressaceae	Wood	Steam distilled	LC:0.69 mg/L	(30)
Cassia obtusifolia	Leguminosae	Seed	Chloroform	$LC_{50:}$ 1.4 ppm	(31)
Cassia tora	Caesulpinaceae	Seed	Methanol	LC_{50}: 20 mg/l	(31)
Piper retrofractum	Piparaceae	Unripe and ripe fruit		LC_{50}:79 ppm	(32)
Curcuma aromatic	Zingiberaceae	Rhizome	Hexane	LC_{50}:36.30 ppm	(33)
Rhinacanthus nasutus	Acanthaceae	Root	Petroleum ether	LC_{50}:3.93 ppm $LC_{90:}$18.51 ppm	(34)
Derris elliptica	Fabaceae	Root	Petroleum ether	LC_{50}:11.17 ppm $LC_{90:}$32.22 ppm	(34)
Homalomena aromatica	Araceae	Whole plant	Petroleum ether	LC_{50}:40.36 ppm $LC_{90:}$77.21 ppm	(34)
Momordica charantia	Cucurbitaceae	Fruit	Hexane	LC_{50}:122.45 ppm LC_{90}:191.86 ppm	(35)
Ocimum basilium	Lamiaceae	Leaf	Water	LC_{50}:.4.66 mg/ml LC_{90}:8.74 mg/ml	(36)
Albizzia amara	Fabaceae	Leaf	Water	LC_{50}:7.10 mg/mi LC_{90}:13.23 mg/ml	(36)
Ageratina adenophora	Asteraceae	Twigs	Acetone	LC_{50}:356.70 ppm	(37)
Millingntonia hortensis	Bignoniaceace	Leaf	Aectone	LC_{50}:104.70 ppm	(38)
Ocimum sanctum	Labiate	Leaf	Acetone	LC_{50}:425.94 ppm	(38)
Citrullus colocynthis	Cucurbitaceae	Leaf	Petroleum ether	LC_{50}: 74.57ppm LC_{90}: 538.30 ppm	(39)
Coccinia indica	Cucurbitaceae	Leaf	Methanol	LC_{50}: 309.46 ppm LC_{90}:1330.43 ppm	(39)
Cucumis sativus	Cucurbitaceae	Leaf	Methanol	LC_{50}: 492.73 ppm LC_{90}: 1824.20 ppm	(39)
Momordica charantia	Cucurbitaceae	Leaf	Methanol	LC_{50}: 199.14 ppm LC_{90}: 780.10 ppm	(39)
Trichosanthes anguina	Cucurbitaceae	Leaf	Acetone	LC_{50}: 554.20 ppm LC_{90}: 2235.34 ppm	(39)
Solanum nigrum	Solanaceae	Dried fruit	Hexane	LC_{50}:17.63 ppm LC_{90}:65.22 ppm	(15)
Fius bengahalensis	Moraceae	Leaf	Methanol	LC_{50}:70.29 ppm LC_{90}:137.23 ppm	(40)
Euodia rideleyi	Rutaceae	Leaf	Ethyl acetate	LC_{50}:139.80 ppm LC_{90}:203.50 ppm	(41)

Table 1: Contd.

Plant species	Plant families	Plant part	Solvent	Lethal concentrations or biological activity	References
Enteromorpha intestinalis	Ulvaceae	Whole plant	Dimethyl sulfoxide (DMSO)	LC_{50}:0.0744 mg/ml LC_{90}:0.1399 mg/ml	(42)
Dictyota dichotoma	Dictyotaceae	Whole plant	Dimethyl sulfoxide (DMSO)	LC_{50}:0.0683 ppm LC_{90}:0.1401 ppm	(42)
Ervatamia coronaria	Apocynaceae	Leaf	Benzene	LC_{50}: 89.59 ppm LC_{90}: 166.04 ppm	(43)
Caesalpinia pulcherrima	Fabaceae	Leaf	Benzene	LC_{50}: 136.36 ppm LC_{90}: 272.15 ppm	(43)
Mentha piperita	Lamiaceae	Essential oil	Ethanol	LC_{50}: 111.9 ppm LC_{90}:295.18 ppm	(44)
Morinda citrifolia	Rubiaceae	Leaf	methanol	LC_{50}: 277.92 ppm LC_{90}:568.18 ppm	(45)
Acalypha alnifolia	Euphorbiaceae	Leaf	methanol	LC_{50}:128.55 ppm LC_{90}:381.67 ppm	(46)
Calotropis gigantean	Asclepiadaceae	Leaf	Ethanol	LC_{50}:136.48 ppm LC_{90}:327.72 ppm	(47)
Citrus sinensis	Rutaceae	Fruit	Ethanol	LC_{50}:342.45 ppm LC_{90}:734.98 ppm	(48)
Aloe vera	Liliaceae	Leaf	Petroleum ether	LC_{50}:253.30 ppm LC_{90}:563.18 ppm	(49)
Sphaeranthus indicus	Asteraceae	Whole plant	Ethyl acetate	LC_{50}: 201.11 ppm LC_{90}: 865.83 ppm	(50)
Citrullus colocynthis	Cucurbitaceae	Whole plant	Dichloromethane	LC_{50}: 515.69 ppm LC_{90}: 1725.59 ppm	(50)
Abutilon indicum	Malvaceae	Leaf	Hexane	LC_{50}: 261.31 ppm LC_{90}:1196.20 ppm	(50)
Cleistanthus collinus	Euphorbiaceae	Leaf	Ethyl acetate	LC_{50}: 560.41 ppm LC_{90}:2669.86 ppm	(50)
Leucas aspera	Lamiaceae	Leaf	Ethyl acetate	LC_{50}: 483.21 ppm LC_{90}:3195.91 ppm	(50)
Murraya koenigii	Rutaceae	Leaf	Diethyl ether	LC_{50}: 511.12 ppm LC_{90}:1882.24 ppm	(50)
Hyptis suaveolens	Lamiaceae	Aerial parts	Hexane	LC_{50}: 543.66 ppm LC_{90}:3546.69 ppm	(50)
Terminalia chebula	Combretaceae	Leaves	Methanol	LC_{50}:93.24 ppm LC_{90}:186.76 ppm	(51)
Senecio laetus	Asteraceae	Root	Methanol	LC_{50}: 22.30 ppm LC_{90}: 144.67 ppm	(52)

Mode of action of phytoextracts

A review on mode of action of phytoextracts revealed that only a few studies have been carried out in this area and there is a lot more to survey (17). However, the studies performed suggest that botanical derivatives have a major effect on the mid gut epithelium and a lesser effect on the gastric area and malpighian tubules (18). Most secondary metabolites of the plants are responsible for the toxic effect which results in insecticidal activity having a wide array of non-specific molecular targets including proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, bio-membranes, and other cellular components (19). Mode and site of action of some of the secondary metabolites of plants have been identified and are listed in this review (Table 3).

Effects of phytoextracts on *Aedes aegypti* other than larvicidal activity

Apart from larvicidal efficacy, some phytoextracts exhibit characteristics which are involved in the inhibition of mosquito growth and reproductive capacity. Some of the foremost physiological characteristics like developmental period, growth, adult emergence, fecundity, fertility and egg hatching are affected by these factors, and hence they are prevalently known as Insect Growth Regulators (IGRs), which can be incorporated in mosquito vector control. Over one thousand plant species contain such compounds including phytoecdysones, anti-juvenile hormones and

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Table 2: Common active compounds of interest isolated from plant species having larvicidal efficacy

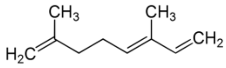
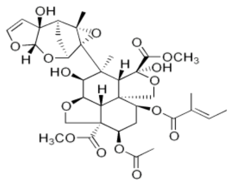
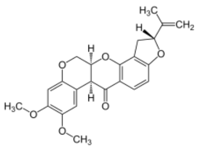
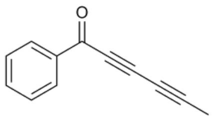
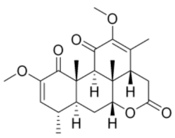
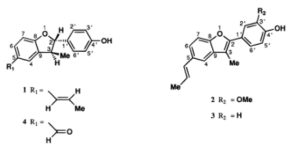
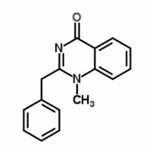
Active Compounds of Interest	Structure	Plant Species	Reference
(5E)-ocimenone		*Tagetes minuta*	(53)
Azadirachtin		*Azadirachta indica*	(54)
Rotenone		*Derris elliptica*	(55)
Capillin		*Artemisia nilagirica*	(56)
Quassin		*Quassia amara*	(57)
Neolignans		*Piper decurrens*	(58)
Arborine		*Glycosmis pentaphylla*	(59)

Table 3: Mode and site of action of some of the secondary metabolites of plants having larvicidal efficacy

Secondary metabolites of plants	Mode and site of action	Reference
Essential oils	Inhibition of acetylecholinestrase	(19)
Thymol	Inhibition of GABA-gated chloride channel	
Pyrethrin	Sodium and potassium ion exchange disruption	
Rotenone	Inhibition of cellular respiration	
Ryanodine	Blockage of calcium channels	
Azadirachtin	Hormonal balance disruption, mitotic poisoning	

phyto-juvenoids, which act as IGRs (20). Some of the important botanical extracts comprising growth and development regulating aptitude are listed in Table 4.

Conclusion

A wide variety of flora around the world has been screened against the major dengue fever vector, *Aedes aegypti*, and identified with their ability to control this vector species either by their insecticidal capability or by other means like growth and development inhibition. These phytoextracts have established their potential and could be constructive in the partial or complete replacement of the chemical insecticides, which presently is the major means of vector control. This will be beneficial, ruling out environmental hazards including harmful effects on non-target organisms. One of the chief challenges that could

Table 4: Effect of selected phytoextracts on growh, development, reproduction, abnormalities, hatch rates and fertility of *Aedes aegypti*

Plant species	Solvent/Fraction/compound	Mg/L	Effect on *Aedes aegypti*	Reference
Azadirachta indica	Water Methyl-tert.-butylether/water	100 20	80.5% pupal – adult intermediates mortality and 3% pupal – adult intermediates exhibited protruding wing-sheath and mouthparts	(60)
Albizzia lebbeck	Ethyl alcohol	500	95% reduced adult emergence at day-7	(61)
Calophyllum inophyllum	Ethyl acetate fraction (seeds)	0.22	EC_{50} for inhibition of adult emergence	(26)
Cassia holosericea	Ethyl alcohol	1000	Prevented pupation up to day-7	(62)
Cyperus iria	Leaf aged 1 & 2 month	1000	100% reduced emergence	(63)
Ervatamia coronaria	Petroleum ether	1000	Prevented pupation up to day-7	(61)
Melia volkensii	Hexane/Ethyl acetate fraction (1:1)	20	100% mortality during molting and 100% reduced emergence	(63)
Azadirachta indica	Oils from crushed seeds	50	Treated larvae developed to pupae but failed to emerge	(24)
Oligochaeta ramose	Acetone	50	74% larval mortality (larval –pupal intermediate) and 76% reduced emergence	(64)
Rhazya stricta	Partially purified asewerine	100	86% emergence at day-7	(65)
Rhinacanthus nasutus	Petroleum ether fraction	2.9	EC_{50} for inhibition of adult emergence	(26)
Solanum suratense,	Ethyl acetate fraction (leaf)	EC50/2	40% decrease in egg hatching	(65)

get eluded is resistance development in the particular vector species.

This review is a preface to the manipulation of vector management strategies using phytoextracts, which is now in its budding stage, but should come to the public interest so that we can conserve our nature without spreading diseases.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' contribution

KVL has done the collection of literature and prepared the content of the manuscript, AVS and EMA provided inputs and supervised the work.

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