

Review Article

Indian J Med Res 135, May 2012, pp 581-598

Plant extracts as potential mosquito larvicides

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Received April 13, 2011

Mosquitoes act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile Virus infection, etc. Under the Integrated Mosquito Management (IMM), emphasis was given on the application of alternative strategies in mosquito control. The continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non target organisms including human health. Application of active toxic agents from plant extracts as an alternative mosquito control strategy was available from ancient times. These are non-toxic, easily available at affordable prices, biodegradable and show broad-spectrum target-specific activities against different species of vector mosquitoes. In this article, the current state of knowledge on phytochemical sources and mosquitocidal activity, their mechanism of action on target population, variation of their larvicidal activity according to mosquito species, instar specificity, polarity of solvents used during extraction, nature of active ingredient and promising advances made in biological control of mosquitoes by plant derived secondary metabolites have been reviewed.

Key words Insecticides - integrated mosquito management - larvicides - LC₅₀ - plant extracts

Introduction

Mosquitoes can transmit more diseases than any other group of arthropods and affect million of people throughout the world. WHO has declared the mosquitoes as “public enemy number one”¹. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population. They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, etc., in almost all tropical and subtropical countries and many other parts of the world.

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and

increasing insecticide resistance on a global scale^{2,3}. Thus, the Environmental Protection Act in 1969 has framed a number of rules and regulations to check the application of chemical control agents in nature⁴. It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco-friendly alternatives such as biological control of vectors has become the central focus of the control programme in lieu of the chemical insecticides.

One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioural and physiological processes. Thus there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases.

Roark⁵ described approximately 1,200 plant species having potential insecticidal value, while Sukumar *et al*⁶ listed and discussed 344 plant species that only exhibited mosquitocidal activity. Shallan *et al* in 2005⁷ reviewed the current state of knowledge on larvicidal plant species, extraction processes, growth and reproduction inhibiting phytochemicals, botanical ovicides, synergistic, additive and antagonistic joint action effects of mixtures, residual capacity, effects on non-target organisms, resistance and screening methodologies, and discussed some promising advances made in phytochemical research. Table I summarized the mosquitocidal activities of various herbal products from edible crops, ornamental plants,

trees, shrubs, herbs, grasses and marine plants according to the extraction procedure developed in eleven different solvent systems and the nature of mosquitocidal activities against different life stages of different vector species as a ready reference for further studies.

Phytochemicals

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920s⁸, but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make upto 1 per cent of world's pesticide market⁹.

Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities⁷. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots, *etc.*, of larger plants or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control.

Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of the plant families-Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various

Table I (A). Efficacy of botanical extracts in controlling/reducing the population of vector mosquitoes

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Petroleum ether solvent extract</i>					
<i>Artemisia annua</i>	Asteraceae	Leaf	<i>Anopheles stephensi</i>	LC ₅₀ value was 16.85 ppm after 24 h and 11.45 ppm after 48 h of exposure	Sharma <i>et al</i> (2006) ¹²
<i>Acacia nilotica</i>	Fabaceae	Leaf		LC ₅₀ value was 55.72 ppm and LC ₉₀ value was 194.58 ppm	Saktivadivel & Daniel (2008) ¹³
<i>Argemone mexicana</i>	Papaveraceae	Leaf, seed		LC ₅₀ value was 30.47 and 24.17; LC ₉₀ values were 246.33 and 184.99 ppm for leaves and seeds respectively	
<i>Jatropha curcas</i>	Euphorbiaceae	Leaf		LC ₅₀ value was 62.29 and LC ₉₀ value was 454.18 ppm	
<i>Withania somnifera</i>	Solanaceae	Leaf		LC ₅₀ value was 65.08 and LC ₉₀ value was 266.39 ppm	
<i>Citrullus colocynthis</i>	Cucurbitaceae	Leaf		LC ₅₀ values were 37.70 and LC ₉₀ value was 52.62 ppm	
<i>Aloe barbadensi</i>	Liliaceae	Leaf		LC ₅₀ values were 29.06 and 22.59 ppm for 24 and 48 h	Maurya <i>et al</i> (2007) ¹⁴
<i>Cannabis sativa</i>	Moraceae	Leaf		LC ₅₀ values were 376.58 and 1316.09 ppm for 24 and 48 h	
<i>Eucalyptus globulus</i>	Myrtaceae	Seed, leaf	<i>Culex pipiens</i>	Both the extracts at a dose of 1000 ppm caused 100 and 80% mortality to the tested larvae	Sheeren <i>et al</i> (2006) ¹⁵
<i>Solanum xanthocarpum</i>	Solanaceae	Root	<i>Cx. pipiens pallens</i>	LC ₅₀ and LC ₉₀ values were 41.28 and 111.16 ppm after 24 h and 38.48 and 80.83 ppm after 48 h, respectively	Mohan <i>et al</i> (2006) ¹⁶
<i>Thymus capitatus</i>	Lamiaceae	Leaf	<i>Cx. pipiens</i>	The volatile oil, Thymol, and the unsaponifiable portion proved high larvicidal potency (LC ₅₀ value was 49.0 ppm)	Mansour <i>et al</i> (2000) ¹⁷
<i>Citrus aurantium</i> ¹⁷	Rutaceae	Fruit peel	<i>Cx. quinquefasciatus</i>	LC ₉₀ values were 53.80 and 32.52 ppm after 24 and 48 h of treatment	Kassir (1989) ¹⁸
<i>Myrtus communis</i>	Myrtaceae	Flower and Leaf	<i>Cx. pipiens molestus</i>	LC ₅₀ value was 16 mg/l. Thymol, carvacrol, (1R)-(+)-pinene and (1S)-(-)-pinene were the most effective toxic compounds with LC ₅₀ values of 36-49 mg/l	Traboulsi <i>et al</i> (2002) ¹⁹
<i>Origanum syriacum</i>	Lamiaceae	Leaf		LC ₅₀ value was 36 mg/l at 24 h of exposure	
<i>Mentha microcarphylla</i>	Anacardiaceae	Leaf		LC ₅₀ value was 39 mg/l at 24 h of exposure	
<i>Pistacia lentiscus</i>	Anacardiaceae	Leaf		LC ₅₀ value was 70 mg/l at 24 h of exposure	
<i>Lavandula stoechas</i>	Lamiaceae	Leaf		LC ₅₀ value was 89 mg/l at 24 h of exposure	
<i>Jatropha curcas</i>	Euphorbiaceae	Leaf	<i>Cx. quinquefasciatus</i>	LC ₅₀ value was 11.34 and LC ₉₀ value was 46.52 ppm	Rahuman <i>et al</i> (2007) ²⁰
<i>Pedilanthus tithymaloides</i>				LC ₅₀ value was 76.61 and LC ₉₀ value was 307.07 ppm	
<i>Phyllanthus amarus</i>				LC ₅₀ value was 113.40 and LC ₉₀ value was 465.28 ppm	

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Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Argemone mexicana</i>	Papaveraceae	Leaf	<i>Cx. quinquefasciatus</i>	Causes 100% mortality at 250 ppm of each extracts	Karmegan <i>et al</i> (1996) ²¹
<i>Jatropha curcus</i>	Euphorbiaceae	Leaf			
<i>Pergularia extensa</i>	Asclepiadaceae	Leaf			
<i>Withania somnifera</i>	Solanaceae	Leaf			
<i>Piper nigrum</i>	Piperaceae	Seed	<i>Cx. pipiens</i>	LC ₅₀ value was 2.6 mg/l	Shaalán <i>et al</i> (2005) ⁷
<i>Euphorbia hirta</i>	Euphorbiaceae	Stem bark	<i>Cx. quinquefasciatus</i>	LC ₅₀ value was 424.94 and LC ₉₀ value was 1314.01 ppm	Rahuman <i>et al</i> (2007) ²²
<i>E. tirucalli</i>	Euphorbiaceae	Stem bark		LC ₅₀ value was 5.52 and LC ₉₀ value was 25.67 ppm	
<i>Ocimum basilicum</i>	Lamiaceae	Leaf	<i>An. stephensi</i> and <i>Cx. quinquefasciatus</i>	LC ₅₀ value of 8.29 and 87.68 ppm respectively	Maurya <i>et al</i> (2009) ²³
<i>Hexane solvent extract</i>					
<i>Momordica charantia</i>	Cucurbitaceae	Fruit	<i>An. stephensi</i>	LC ₅₀ value was 0.50 and LC ₉₀ value was 1.54% concentration of the extract	Singh <i>et al</i> (2006) ²⁴
			<i>Cx. quinquefasciatus</i>	LC ₅₀ value was 1.29 and LC ₉₀ value was 4.11% concentration of the extract	
			<i>Ae. aegypti</i>	LC ₅₀ value was 1.45 and LC ₉₀ value was 4.46% concentration of the extract	
<i>Kaempferia galanga</i>	Zingiberaceae	Rhizome	<i>Cx. quinquefasciatus</i>	LC ₅₀ value was 42.33 ppm	Choochote <i>et al</i> (1999) ²⁵
<i>Khaya senegalensis</i>	Meliaceae	Leaf	<i>Cx. annulirostris</i>	LC ₅₀ value was 5.86 mg/l	Shaalán <i>et al</i> (2005) ⁷
<i>Daucus carota</i>	Apiaceae	Leaves		LC ₅₀ value was 77.19 mg/l	
<i>Curcuma aromatica</i>	Zingiberaceae	Rhizome	<i>Ae. aegypti</i>	LC ₅₀ value was 36.30 ppm	Choochate <i>et al</i> (2005) ²⁶
<i>Cybistax antisiphilitica</i>	Bignoniaceae	Stem wood	<i>Ae. aegypti</i>	A natural quinone identified as 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (lapachol) was quite potent with LC ₅₀ value 26.3 µg/ml	Rodrigues <i>et al</i> (2005) ²⁷
<i>Eucalyptus citriodora</i>	Myrtaceae	Leaf	<i>An. stephensi</i> , <i>Cx. quinquefasciatus</i> , <i>Ae. aegypti</i>	The LC ₅₀ values against IVth instar larvae of three species were 69.86, 81.12 & 91.76 ppm, respectively after 24 h and 26.7, 29.9 & 38.8 ppm, respectively after 72 h	Singh <i>et al</i> (2007) ²⁸
<i>Solanum nigrum</i>	Solanaceae	Dried fruit	<i>An. Culicifacies</i> , <i>An. stephensi</i> , <i>Cx. quinquefasciatus</i> , <i>Ae. aegypti</i>	The LC ₅₀ values against IVth instar larvae of four species were 9.04, 6.25, 12.25 and 17.63 ppm respectively	Raghavendra <i>et al</i> (2009) ²⁹

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Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Acetone solvent extract</i>					
<i>Tridax procumbens</i>	Compositae	Leaf	<i>An. subpictus</i>	LC ₅₀ value of 39.98 mg/l	Kamaraj <i>et al</i> (2011) ³⁰
<i>Ageratum conyzoides</i>	Asteraceae	Leaf	<i>Cx. quinquefasciatus</i>	Potent larvicidal activity was noticed	Saxena <i>et al</i> (1992) ³¹
<i>Cleome icosandra</i>	Capparaceae	Leaf			
<i>Tridax procumbens</i>	Compositae	Leaf			
<i>Ageratina adenophora</i>	Asteraceae	Twigs	<i>Ae. aegypti</i> and <i>Cx. quinquefasciatus</i>	At 24 h, LC ₅₀ value of the extract was found to be 356.70 ppm for <i>Ae. aegypti</i> and 227.20 ppm for <i>Cx. quinquefasciatus</i>	Raj Mohan & Ramaswamy (2007) ³²
<i>Feronia limonia</i>	Rutaceae	Leaf	<i>Cx. quinquefasciatus</i> , <i>An. stephensi</i> , <i>Ae. aegypti</i>	LC ₅₀ values of 129.24, 79.58 and 57.23 ppm for three mosquito species respectively	Rahuman <i>et al</i> (2000) ³³
<i>Millingtonia hortensis</i>	Bignoniaceae	Leaf	<i>An. stephensi</i> , <i>Ae. aegypti</i> and <i>Cx. quinquefasciatus</i>	LC ₅₀ values of 104.70, 138 and 83.18 ppm for 2 nd instar larvae of three species at 24 h of bioassay	Kaushik & Saini (2008) ³⁴
<i>O. sanctum</i>	labiate	Leaf	<i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i>	The LC ₅₀ values of <i>O. sanctum</i> against the larvae of <i>Ae. aegypti</i> was 425.94, and against the larvae of <i>Cx. quinquefasciatus</i> was 592.60 ppm	Anees (2008) ³⁵
<i>Carbon tetra chloride solvent extract</i>					
<i>Aloe barbadensis</i>	Liliaceae	Leaf	<i>An. stephensi</i>	LC ₅₀ values of 15.58 and 8.04 ppm after 24 and 48 h of exposure, respectively	Maurya <i>et al</i> (2007) ¹⁴
<i>S. xanthocarpum</i>	Solanaceae	Root	<i>Cx. pipiens pallens</i>	LC ₅₀ and LC ₉₀ values were 64.99 and 252.43 ppm and 59.20 and 186.15 ppm after 24 and 48 h of exposure, respectively	Mohan <i>et al</i> (2006) ¹⁶
<i>E. globulus</i>	Myrtaceae	Seed and leaf	<i>Cx. pipiens</i>	Both the extracts at a dose of 1000 ppm caused 100 and 80% mortality to the tested larvae	Sheeren (2006) ¹⁵
<i>Chloroform extract</i>					
<i>Plumbago zeylanica</i> , <i>P. dawei</i> and <i>P. stenophylla</i>	Plumbaginaceae	Root	<i>An. gambiae</i>	LC ₅₀ values were 4.1, 6.4 and 6.7 mg/ml respectively. LC ₉₀ values were 10.6, 26.2 and 15.6 mg/ml, respectively	Maniafu <i>et al</i> (2009) ³⁶
<i>Euphorbia tirucalli</i>	Euphorbiaceae	Latex and stem bark	<i>Cx. pipiens pallens</i>	LC ₅₀ value was 200.76 and LC ₉₀ value was 343.515 mg/l	Yadav <i>et al</i> (2002) ³⁷
<i>Nyctanthes arbortristis</i>	Nyctantheceae	Flower	<i>Cx. quinquefasciatus</i>	LC ₅₀ values were 25.67, 22.19; 38.60, 28.95; 53.14, 42.14 and 72.60, 61.82 ppm and for the isolated compound NCS-2 were 73.31, 65.48; 83.02, 67.02; 97.26, 81.84 and 14.68, 99.02 ppm for 1 st , 2 nd , 3 rd and 4 th instar larvae, respectively at 24 and 48 h post-exposure	Khatune <i>et al</i> (2001) ³⁸

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Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Citrus sinensis</i>	Rutaceae	Fruit peel	<i>An. subpictus</i>	LC ₅₀ value was 58.25 and LC ₉₀ value was 298.31 ppm	Bagavan <i>et al</i> (2009) ³⁹
<i>Aloe ngongensis</i>	Asphodelaceae	Leaf	<i>An. gambie</i>	LC ₅₀ value was 58.25 mg/ml	Matasyoh <i>et al</i> (2008) ⁴⁰
<i>Millettia dura</i>	Leguminosae	Seed	<i>Ae. aegypti</i>	Rotenoids, deguelin and tephrosin, isolated from the seeds of this plant showed potent activities, with LC ₅₀ values of 1.6 and 1.4 µg/ml at 24 h, respectively	Yenesew <i>et al</i> (2003) ⁴¹
<i>Cassia obtusifolia</i>	Leguminosae	Seed	<i>Ae. aegypti</i> , <i>Ae. togoi</i> , and <i>Cx. pipiens pallens</i>	Showed a strong larvicidal activity of 100% mortality at 25 mg/l. The biologically active component was emodin. The LC ₅₀ values of emodin were 1.4, 1.9, and 2.2 ppm respectively	Yang <i>et al</i> (2003) ⁴²
<i>Methanol extract</i>					
<i>Atlantia monophylla</i>	Rutaceae	Leaf	<i>An. stephensi</i>	LC ₅₀ value of 0.05 mg/l. Insect growth regulating activity with EI ₅₀ value 0.065 mg/l	Sivagnaname & Kalyanasundaram (2004) ⁴³
<i>Dysoxylum malabaricum</i>	Meliaceae	Leaf	<i>An. stephensi</i>	4% concentration of leaf extract killed more than 97% of first instars, 92% of fifth instars, 93% of pupae and 91% of adults	Senthil Nathan <i>et al</i> (2006) ⁴⁴
<i>Melia azedarach</i>	Meliaceae	Leaf and seeds	<i>An. stephensi</i>	The extract showed strong larvicidal activity	Senthil Nathan <i>et al</i> (2006) ⁴⁵
<i>Moringa oleifera</i>	Moringaceae	Bark	<i>Cx. gelidus</i>	LC ₅₀ value was 38.47 µg/ml	Kamaraj & Rahuman (2010) ⁴⁶
<i>Ocimum gratissimum</i>	Lamiaceae	Leaf	<i>Cx. gelidus</i>	LC ₅₀ value was 21.83 µg/ml	Kamaraj & Rahuman (2010) ⁶²
<i>Solenostemma argel</i>	Apocynaceae	Aerial parts	<i>Cx. pipiens</i>	LC ₅₀ values of 0.037, 0.031, 0.009 and 0.007 ppm and the LC ₉₅ values were found as 0.394, 0.293, 0.065 and 0.030 ppm, after 1, 2, 4 and 7 days against the larva of <i>Cx. pipiens</i> under laboratory conditions	Al-Doghairi <i>et al</i> (2004) ⁴⁷
<i>S. xanthocarpum</i>	Solanaceae	Root	<i>Cx. pipiens pallens</i>	LC ₅₀ and LC ₉₀ were 248.55 and 578.25 ppm and 215.52 and 562.72 ppm after 24 and 48 h of exposure, respectively	Mohan <i>et al</i> (2006) ¹⁶
<i>Chrysanthemum indicum</i>	Asteraceae	Leaf	<i>Cx. tritaeniorhynchus</i>	LC ₅₀ value was 42.29 mg/ml after 24 h	Kamaraj <i>et al</i> (2010) ⁴⁸

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Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Azadirachta indica</i>	Meliaceae	Leaf	<i>Cx. pipens</i>	Showed an acute and chronic LC ₅₀ and 95% CL at 824 and 265 ppm	El Hag <i>et al</i> (1999) ⁶⁵
<i>Rhazya stricta</i>	Apocynaceae	Leaf		Acute (2 d) and chronic (10 d) toxic effects, having an LC ₅₀ and 95% CL at 251 and 140 ppm	
<i>Momordica charantia</i>	Cucurbitaceae	Leaf	<i>Cx. quinquefasciatus</i>	LC ₅₀ value was 465.85; LC ₉₀ value was 2421.46 ppm	Prabakar & Jebanesan (2004) ⁴⁹
<i>Trichosanthes anguina</i>				LC ₅₀ value was 567.81; LC ₉₀ value was 2915.48 ppm	
<i>Luffa acutangula</i>				LC ₅₀ value was 839.81; LC ₉₀ value was 3286.25 ppm	
<i>Benincasa cerifera</i>				LC ₅₀ value was 1189.30; LC ₉₀ value was 6528.5 ppm	
<i>Citrullus vulgaris</i>				LC ₅₀ value was 1636.04; LC ₉₀ value was 11473.92 ppm	
<i>Vitex negundo</i>	Verbenaceae	Leaf	<i>Cx. quinquefasciatus</i>	LC ₅₀ value was 212.57 ppm	Krishnan <i>et al</i> (2007) ⁵⁰
<i>V. trifolia</i>				LC ₅₀ value was 41.41 ppm	
<i>V. peduncularis</i>				LC ₅₀ value was 76.28 ppm	
<i>V. altissima</i>				LC ₅₀ value was 128.04 ppm	
<i>Centella asiatica</i>	Umbelliferae	Leaf	<i>Cx. quinquefasciatus</i>	LC ₅₀ ranged between 6.84 ppm at 19°C and 1.12 ppm at 31°C. LC ₉₀ varied from 9.12 to 3.63 ppm at the two temperatures, respectively	Rajkumar & Jebanesan (2005) ⁵¹
<i>Euphorbia tirucalli</i>	Euphorbiaceae	Latex and stem bark	<i>Cx. pipiens pallens</i>	LC ₅₀ value was 177.14; LC ₉₀ value was 513.387 mg/l	Yadav <i>et al</i> (2002) ³⁷
<i>Eucalyptus globulus</i>	Myrtaceae	Seed and leaf	<i>Cx. pipiens</i>	At a dose of 1000 ppm caused 100% mortality of the tested larvae	Sheeren (2006) ¹⁵
<i>Atlantia monophylla</i>	Rutaceae	Leaf	<i>Cx. quinquefasciatus</i>	Larvae were found susceptible with LC ₅₀ value of 0.14 mg/l	Sivagnaname & Kalyanasundaram (2004) ⁴³
<i>Pavonia zeylanica</i>	Malvaceae	Leaf	<i>Cx. quinquefasciatus</i>	After 24 h of treatment the LC ₅₀ values was 2214.7 ppm	Vahitha <i>et al</i> (2002) ⁵²
<i>Acacia ferruginea</i>	Leguminosae	Leaf	<i>Cx. quinquefasciatus</i>	After 24 h of treatment the LC ₅₀ value was 5362.6 ppm	
<i>Coccinia indica</i> , <i>Cucumis sativus</i> , <i>Momordica charantia</i>	Cucurbitaceae	Leaf	<i>Cx. quinquefasciatus</i> and <i>Ae. aegypti</i>	LC ₅₀ values of the respective plants were, 377.69, 623.80, 207.61 and 309.46, 492.73 and 199.14 ppm against the two vector species	Rahuman & Venkatesan (2008) ²²
<i>Cassia tora</i>	Caesulpinaceae	Seed	<i>Ae. aegypti</i> and <i>Cx. pipiens pallens</i>	LC ₅₀ value was 20mg/l for both the larval species	Jang <i>et al</i> (2002) ⁷³

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Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Atlantia monophylla</i>	Rutaceae	Leaf	<i>Ae. aegypti</i>	Larval growth regulating activity of this extract was found to be pronounced with EI ₅₀ value 0.002 mg/l	Sivagnaname & Kalyanasundaram (2004) ⁴³
<i>Coccinia indica</i> , <i>Cucumis sativus</i> , <i>Momordica charantia</i>	Cucurbitaceae	Leaf	<i>Ae. albopictus</i>	LC ₅₀ value was 309.46, 492.73 and 199.14 ppm respectively	Rahuman & Venkatesan (2008) ²²
<i>Aristolochia saccata</i>	Aristolochiaceae	Root		LC ₅₀ value was 14.52; LC ₉₀ value was 42.68 ppm	Das <i>et al</i> (2007) ⁵⁴
<i>Annona squamosa</i>	Annonaceae	Leaf		LC ₅₀ value was 20.26; LC ₉₀ value was 86.59 ppm	
<i>Gymnopetalum cochinchinensis</i>	Cucurbitaceae	Fruit/ pericarp		LC ₅₀ value was 50.67; LC ₉₀ value was 155.12 ppm	
<i>Caesalpinea</i> sp.	Leguminosae	Bark		LC ₅₀ value was 53.66; LC ₉₀ value was 169.41 ppm	
<i>Piper</i> sp.	Piperaceae	Stem		LC ₅₀ value was 144.22; LC ₉₀ value was 357.32 ppm	
<i>Chamaecyparis obtusa</i>	Cupressaceae	Leaf	<i>An. stephensi</i>	The bioactive component in the leaf extract was characterized as beta-thujaplicin by spectroscopic analyses. The LC ₅₀ value of beta-thujaplicin was 2.91 ppm	Jang <i>et al</i> (2005) ⁵⁵
<i>Acalypha alnifolia</i>	Euphorbiaceae	Leaf	<i>An. stephensi</i> , <i>Ae. aegypti</i> and <i>Cx. quinquefasciatus</i>	LC ₅₀ values were 125.73, 127.98 and 128.55 ppm against 4 th instar larvae of three mosquito species at 24 h	Kovendan <i>et al</i> (2012) ⁵⁶
<i>Chloroform: methanol extract(1:1)</i>					
<i>Solanum villosum</i>	Solanaceae	Leaf	<i>An. subpictus</i>	LC ₅₀ values for all instars were between 24.20 and 33.73 ppm after 24 h and between 23.47 and 30.63 ppm after 48 h of exposure period	Chowdhury <i>et al</i> (2009) ⁵⁷
<i>Cestrum diurnum</i>	Solanaceae	Leaf	<i>An. stephensi</i>	The LC ₅₀ value of the active ingredient was determined as 0.70, 0.89, 0.90 and 1.03mg/100mL, for 1 st , 2 nd , 3 rd and 4 th instar larva respectively in 24 h study period	Ghosh & Chandra (2006) ⁵⁸
			<i>Cx. quinquefasciatus</i>	LC ₅₀ value of 0.29, 0.35, 0.57 and 0.65% for 1 st , 2 nd , 3 rd and 4 th instar larvae at 24 h	Ghosh <i>et al</i> (2008) ⁵⁹
<i>Solanum villosum</i>	Solanaceae	Berry	<i>Ae. aegypti</i>	LC ₅₀ value of 5.97 ppm at 72 h of bioassay	Chowdhury <i>et al</i> (2008) ⁶⁰
<i>Ethanol Extract</i>					
<i>Cassia obtusifolia</i>	Leguminosae	Leaf	<i>An. stephensi</i>	LC ₅₀ and LC ₉₀ values were 52.2 and 108.7 mg/l	Rajkumar & Jebanesan (2009) ⁶¹
<i>Azadirachta indica</i>	Meliaceae	Leaf	<i>Cx. fatigans</i>	In comparison with malathion (LC ₅₀ value was 0.45 ppm) the LC ₅₀ value of neem fraction (NLX) was found to be higher to the third instar larvae at 390 ppm	Azmi <i>et al</i> (1998) ⁶²

Contd....

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Piper retrofractum</i>	Piperaceae	Unripe and ripe fruit	<i>Cx. quinquefasciatus</i>	The ripe fruit extract (002/3) was somewhat less active than ripe fruit extract (001/4) with lesser larvicidal activity	Chansang <i>et al</i> (2005) ⁶³
<i>Citrus reticulata</i>	Rutaceae	Seed	<i>Cx. quinquefasciatus</i> and <i>Ae. aegypti</i>	LC ₅₀ value against <i>Ae. aegypti</i> and <i>Cx. quinquefasciatus</i> larvae was 2,267.71, and 2,639.27 ppm respectively	Sumroiphon <i>et al</i> (2006) ⁶⁴
<i>Azadirachta indica</i>	Meliaceae	Leaf	<i>Ae. aegypti</i>	LC ₅₀ value is 8.32 mg/ml	Mgbemena (2010) ⁶⁵
<i>Azadirachta indica</i> , <i>Ocimum gratissimum</i> and <i>Citrus citratus</i>	Meliaceae, Lamiaceae and Rutaceae respectively	Leaf	<i>Ae. aegypti</i>	<i>A. indica</i> showing the greatest toxicity having LC ₅₀ at 8.32mg/ml, while on the other hand <i>O. gratissimum</i> and <i>C. citratus</i> had LC ₅₀ 19.50mg/ml and 34.67mg/ml respectively	Mgbemena (2010) ⁶⁵
<i>Apium graveolens</i>	Umbelliferae	Seed	<i>Ae. aegypti</i>	LD ₅₀ and LD ₉₅ values of 81.0 and 176.8 mg/l, respectively for fourth instar larvae	Choochate <i>et al</i> (2004) ⁶⁶
<i>Rhizophora mucronata</i>	Rhizophoraceae	Bark, pith, stem wood	<i>Ae. aegypti</i>	LC ₅₀ values of 157.4, 168.3 and 1003.4 ppm for bark, pith and stem wood at 48 h respectively	Kabaru & Gichia (2001) ⁶⁷
<i>Piper longum</i>	Piperaceae	Fruit exocarp	<i>Ae. aegypti</i>	LC ₅₀ value of 2.23 ppm	Chaithong <i>et al</i> (2006) ⁶⁸
<i>P. ribesoides</i>	Piperaceae	Fruit exocarp	<i>Ae. aegypti</i>	LC ₅₀ value of 8.13 ppm	
<i>P. sarmentosum</i>	Piperaceae	Fruit exocarp	<i>Ae. aegypti</i>	LC ₅₀ value of 4.06 ppm	
<i>Annona crassiflora</i>	Annonaceae	Root wood	<i>Ae. aegypti</i>	LC ₅₀ value was 0.71; LC ₉₀ value was 5.12 µg/ml	Omena <i>et al</i> (2007) ⁶⁹
		Root bark		LC ₅₀ value was 8.94; LC ₉₀ value was 39.00 µg/ml	
		Stem		LC ₅₀ value was 16.1; LC ₉₀ value was 54.8 µg/ml	
<i>A. glabra</i>	Annonaceae	Seed		LC ₅₀ value was 0.06; LC ₉₀ value was 2.75 µg/ml	
<i>A. muricata</i>	Annonaceae	Root		LC ₅₀ value was 42.3; LC ₉₀ value was 200 µg/ml	
<i>A. squamosa</i>	Annonaceae	Root		LC ₅₀ value was 31.9; LC ₉₀ value was 66.2 µg/ml	
		Leaf		LC ₅₀ value was 169; LC ₉₀ value was 748 µg/ml	
<i>Denis</i> sp.	Leguminoseae	Root		LC ₅₀ value was 8.54; LC ₉₀ value was 15.2 µg/ml	
<i>Erythrina mulungu</i>	Leguminoseae	Stem bark		LC ₅₀ value was 67.9; LC ₉₀ value was 15.2 µg/ml	
<i>Pterodon polygalaeflorus</i>	Leguminoseae	Seed		LC ₅₀ value was 35.7; LC ₉₀ value was 63.6 µg/ml	
<i>Tagetes minuta</i>	Asteraceae	Aerial parts	<i>Ae. fluviatilis</i>	LC ₉₀ of 1.5 mg/l and LC ₅₀ of 1.0 mg/l.	Macedo <i>et al</i> (1997) ⁷⁰

Contd....

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Eclipta paniculata</i>	Asteraceae	Aerial parts	<i>Ae. fluviatilis</i>	LC ₉₀ of 17.2 mg/l and LC ₅₀ of 3.3 mg/l	
<i>Benzene extract</i>					
<i>Citrullus vulgaris</i>	Cucurbitaceae	Leaf	<i>Ae. stephensi</i>	100% mortality was exerted at 250 ppm and the corresponding LC ₅₀ value was 18.56 ppm	Mullai <i>et al</i> (2008) ⁷¹
<i>Acalypha indica</i>	Euphorbiaceae	Leaf	<i>An. stephensi</i>	LC ₅₀ value was 19.25 ppm at 24 h	Govindarajan <i>et al</i> (2008) ⁷²
<i>C. vulgaris</i>	Cucurbitaceae	Leaf	<i>Ae. aegypti</i>	LC ₅₀ value was 42.76 ppm	Mullai <i>et al</i> (2008) ⁷³
<i>Ethyl acetate extract</i>					
<i>Dysoxylum malabaricum</i>	Meliaceae	Leaf	<i>An. stephensi</i>	Fourth instars were more susceptible to the extract when compared with pupae and adults	Senthil Nathan <i>et al</i> (2008) ⁷⁴
<i>D. beddomei</i>					
<i>Aloe turkanensis</i>	Asphodelaceae	Leaf	<i>An. gambiae</i>	100% mortality was achieved at a concentration of 0.2 mg/ml and it had a LC ₅₀ value of 0.11mg/ml	Matasyoh <i>et al</i> (2008) ⁴⁰
<i>Solanum nigrum</i>	Solanaceae	Leaf	<i>Cx. quinquefasciatus</i>	LC ₅₀ value was 17.04 ppm against 4 th instar larvae after 24 h	Rawani <i>et al</i> (2010) ⁷⁵
<i>Ocimum gratissimum</i>	Lamiaceae	Leaf	<i>Cx. gelidus</i> and <i>Cx. quinquefasciatus</i>	LC ₅₀ values were 39.31 and 66.28 µg/ml against 4 th instar larvae after 24 h	Kamaraj & Rahuman (2010) ⁴⁸
<i>Annona squamosa</i>	Annonaceae	Bark	<i>Cx. quinquefasciatus</i> and <i>An. stephensi</i>	LC ₅₀ values of 28.18 and 43.07 ppm against <i>An. stephensi</i> and <i>Cx. quinquefasciatus</i> respectively	Kamaraj <i>et al</i> (2010) ⁴⁸
<i>O. sanctum</i>	Labiates	Leaf	<i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i>	The LC ₅₀ values of <i>O. sanctum</i> against the larvae of <i>Ae. aegypti</i> was 425.94, and against the larvae of <i>Cx. quinquefasciatus</i> was 592.60 ppm	Anees (2008) ³⁵
<i>Aqueous extract</i>					
<i>Carica papaya</i>	Caricaceae	Seed	<i>Cx. quinquefasciatus</i>	LC ₅₀ value of 0.15, 0.11, 0.07 and 0.20 % against 1 st , 2 nd , 3 rd and 4 th instar larvae	Rawani <i>et al</i> (2009) ⁷⁶
<i>Murraya paniculata</i>	Rutaceae	Fruit		LC ₅₀ value of 0.05, 0.06, 0.08 and 0.31% against 1 st , 2 nd , 3 rd and 4 th instar larvae	
<i>Cleistanthus collinus</i>	Euphorbiaceae	Leaf		LC ₅₀ value of 0.21, 0.27, 0.29 and 0.40 % against 1 st , 2 nd , 3 rd and 4 th instar larvae	
			<i>An. gambiae</i>	LC ₅₀ value was 409.77 and LC ₉₀ value was 831.08 ppm	
<i>Hemidesmus indicus</i>	Asclepiadaceae	Root	<i>Cx. quinquefasciatus</i>	80% mortality was observed in 5% concentration after 1 day of exposure	Khanna & Kannabiran (2007) ⁷⁷
<i>Gymnema sylvestre</i>	Asclepiadaceae	Leaf	<i>Cx. quinquefasciatus</i>	6.6% mortality was observed in 5% concentration after 1 day of exposure	
<i>Eclipta prostrata</i>	Asteraceae	Leaf, root	<i>Cx. quinquefasciatus</i>	78.3% mortality was observed in 5% concentration after 1 day of exposure	

Contd....

Plant species	Family	Plant parts used	Target mosquito species	Lethal concentrations/ biological activity	References
<i>Artimisia cina</i>	Compositaeae	Leaf	<i>Cx. pipens</i>	The EC ₅₀ for the mosquito at 24 h after treating with extract was 4.0 g/l	Aly & Bardan (1986) ⁷⁸
<i>Cleome droserifolia</i>	Capparidaceae	Leaf	<i>Cx. pipens</i>	The EC ₅₀ for the mosquito at 24 h after treating with extract was 4.7 g/l	
<i>Piper retrofractum</i>	Piperaceae	Un ripe and ripe fruit	<i>Cx. quinquefasciatus</i> and <i>Ae. aegypti</i>	LC ₅₀ value of 135 against <i>Cx. quinquefasciatus</i> and 79 ppm against <i>Ae. aegypti</i>	Chansang <i>et al</i> (2005) ⁶³
<i>Solanum villosum</i>	Solanaceae	Leaf	<i>An. stephensi</i> , <i>Cx. quinquefasciatus</i> and <i>Ae. aegypti</i>	The protein compound responsible for mosquitocidal property was isolated with a LC ₅₀ value of 644.75, 645.75 and 747.22 ppm	Chowdhury <i>et al</i> (2008) ⁷⁹
<i>Solanum nigrum</i>	Solanaceae	Dried fruit	<i>An. culicifacies</i> species A, <i>An. culicifacies</i> species C, <i>An. stephensi</i> , <i>Cx. quinquefasciatus</i> and <i>Ae. aegypti</i>	The LC ₅₀ of <i>An. culicifacies</i> species A was the lowest while that of <i>Ae. aegypti</i> was highest in the order, <i>An. culicifacies</i> species A (208.5 ppm) > <i>An. stephensi</i> (242.5 ppm) > <i>An. culicifacies</i> species C (251.7 ppm) > <i>Cx. quinquefasciatus</i> (337.2 ppm) > <i>Ae. aegypti</i> (359 ppm)	Raghavendra <i>et al</i> (2009) ³⁹
<i>Steam distillation</i>					
<i>Paullinia clavigera</i>	Sapindaceae	Leaf	<i>An. benarrochi</i>	LC ₅₀ (24 h) value was 0.81; LC ₅₀ (12 h) value was 1.19%	Iannacone & Pérez (2004) ⁸⁰
<i>Tradescintia zebrina</i>	Commelinaceae		<i>An. benarrochi</i>	LC ₅₀ (24 h) value was 0.81; LC ₅₀ (12 h) value was 7.83%	

types of larval, adulticidal or repellent activities against different species of mosquitoes⁷.

Application of phytochemicals as mosquito larvicide: An essential component of IMM

Human beings have used plant parts, products and secondary metabolites of plant origin in pest control since early historical times. Vector control has been practiced since the early 20th century. During the pre-DDT era, reduction of vector mosquitoes mainly depended on environmental management of breeding habitats, *i.e.*, source reduction. During that period, some botanical insecticides used in different countries were Chrysanthemum, Pyrethrum, Derris, Quassia, Nicotine, Hellebore, Anabasine, Azadirachtin, d-limonene camphor, Turpentine, *etc*⁷.

From the early 1950s, DDT and other synthetic organochloride and organophosphate insecticides were extensively used to interrupt transmission of vector borne diseases by reducing densities, human-vector

contact and, in particular, the longevity of vector mosquitoes. In the mid-1970s, the resurgence of vector borne diseases, along with development of insecticide resistance in vector population, poor human acceptance of indoor house spraying and environmental concerns against the use of insecticides led to a rethinking in vector control strategies¹⁰. As a result, emphasis was given on the application of alternative methods in mosquito control as part of the Integrated Mosquito Management (IMM)¹¹. Integrated Mosquito Management (IMM) is a decision-making process for the management of mosquito populations, involving a combination of methods and strategies for long-term maintenance of low levels of vectors. The purpose of IMM is to protect public health from diseases transmitted by mosquitoes, maintain healthy environment through proper use and disposal of pesticides and improve the overall quality of life through practical and effective pest control strategies. The main approaches of IMM include: (i) Source reduction and habitat management

by proper sanitation, water management in temporary and permanent water bodies, and channel irrigation. Vegetation management is also necessary to eliminate protection and food for mosquito larvae; (ii) Larviciding by application of dipteran specific bacteria, insect growth regulators, surface films and oils, expanded polystyrene beads, phytochemicals, organophosphates and organochlorides, (iii) Adulticiding by application of synthetic pyrethroids, organophosphates and synthetic or plant derived repellents, insecticide impregnated bed nets, genetic manipulations of vector species, etc., (iv) Use of mosquito density assessment in adult and larval condition and disease surveillance; and (v) Application of biological control methods by using entomophagous bacteria, fungi, microsporidians, predators and parasites.

Of the above avenues of IMM, larviciding approach is the more proactive, proenvironment, target specific and safer approach than controlling adult mosquitoes. Application of larvicide from botanical origin was extensively studied as an essential part of IMM, and various mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin were developed⁷.

Variation of larvicidal potentiality according to mosquito species, plant parts and polarity of solvents used

The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species. Sukumar *et al*⁶ have described the existence of variations in the level of effectiveness of phytochemical compounds on target mosquito species *vis-à-vis* plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction. Changes in the larvicidal efficacy of the plant extracts occurred due to geographical origin of the plant (in *Citrus* sp^{18,39,64,65}, *Jatropha* sp^{13,20,21}, *Ocimum sanctum*^{22,35,65,82}, *Momordica charantia*^{22,24,49}, *Piper* sp^{54,63,89,95} and *Azadirachta indica*⁶⁵); response in the different mosquito species (in *Curcuma domestica*²⁶, *Withania somnifera*¹³, *Jatropha curcas*^{13,20}, *Piper retrofractum*⁶³, *Cestrum diurnum*⁵⁸, *Citrullus vulgaris*^{50,71}, and *Tridax procumbens*^{30,31}); due to variation in the species of plant examined (in *Euphorbia* sp^{22,28,37,51}, *Phyllanthus*

sp²⁰, *Curcuma* sp³⁶, *Solanum* sp^{16,29,57,60,75,79,96}, *Ocimum* sp^{23,35,65,82}, *Eucalyptus* sp^{22,28,37,51}, *Plumbago* sp²⁰, *Vitex* sp^{50,93}, *Piper* sp^{54,63,89,95}, *Annona* sp^{48,54,69}, and *Cleome* sp^{31,78}) and between plant parts used to study the larvicidal efficacy (in *Euphorbia tirucalli*^{28,51}, *Solanum xanthocarpum*¹⁶, *Azadirachta indica*⁶⁵, *Solanum villosum*^{57,60,79,96}, *Annona squamosa*^{48,54,69}, *Withania somnifera*¹³, *Melia azedarach*⁴⁵, *Moringa oleifera*⁴⁶ and *Ocimum sanctum*^{35,82}). However, the principal objective of the present documentation is to report the changes in larvicidal potentiality of the plant extracts due to change of the particular solvent used during extraction. Variation of the larvicidal potential of the same plant changed with the solvents used as evidenced in case of *Solanum xanthocarpum*¹⁶, *Euphorbia tirucalli*^{28,51}, *Momordica charantia*^{22,24,49}, *Eucalyptus globules*^{14,15,28,83}, *Citrullus colocynthis*¹³, *Azadirachta indica*⁶⁵, *Annona squamosa*^{48,54,69} and *Solanum nigrum*^{29,75}.

It has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents used. Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules. This was achieved by using mainly eleven solvent systems ranging from hexane/ petroleum ether, the most non polar (polarity index of 0.1 that mainly extracts essential oil) to that of water, the most polar (polarity index of 10.2) that extracts biochemical with higher molecular weights such as proteins, glycans, etc. Chloroform or ethyl acetate are moderately polar (polarity index of 4.1) that mainly extracts steroids, alkaloids, etc. It has been found that in most of the studies solvent with minimum polarity have been used such as hexane or petroleum ether or that with maximum polarity such as aqueous/ steam distillation. However, those biochemical that were extracted using moderately polar solvents were also seen to give good results as reported by a few bioassay. Thus, different solvent types can significantly affect the potency of extracted plant compounds and there is difference in the chemo-profile of the plant species. In Table I, the lowest LC₅₀ value was reported in *Solenostemma argel* against *Cx. pipiens*⁴⁷. Several other plants such as *Nyctanthes arbotristis*³⁸, *Atlantia monophylla*⁵⁷, *Centella asiatica*⁴⁰, *Cryptotaenia paniculata*⁷⁶ were also reported with promising LC₅₀ values. These extracts may be fractioned in order to locate the particular bioactive toxic agent responsible for larval toxicity. Table I also reported that most of the studies were carried out on *Culex* mosquitoes and *Aedes* was the least frequently chosen mosquitoes for all the experiments. In several studies, instead of a particular

Table II. Identification of various bioactive toxic principles from plant extract and their relative mosquitocidal efficacy

Active ingredient	Mosquito	Plants	LC/LD values	References
Octacosane	<i>Cx. quinquefasciatus</i>	<i>Moschosma polystachyum</i>	LC ₅₀ value of 7.2±1.7 mg/l	Rajkumar & Jebanesan (2004) ⁸¹
(E)-6-hydroxy-4,6-dimethyl-3-heptene-2-one	<i>Ae. aegyptii</i>	<i>Ocimum sacnctum</i>	LD ₁₀₀ value of 6.25 µg/ml	Kelm & Nair (1998) ⁸²
α-terpinene	<i>Ae. aegyptii</i>	<i>Eucalyptus camaldulensis</i>	LC ₅₀ value of 14.7 µg/mL	Jantan <i>et al</i> (2005) ⁸³
Geranial	<i>Ae. aegyptii</i>	<i>Magnolia salicifolia</i>	LD ₁₀₀ value of 100 ppm	Kelm <i>et al</i> (1997) ⁸⁴
Germacrene D	<i>An. gambiae</i> , <i>Cx. quinquefasciatus</i> and <i>Ae. aegyptii</i>	<i>Chloroxylon swietenia</i>	LD ₅₀ values of 1.8, 2.1 and 2.8×10 ⁻³	Kiran and Devi (2007) ⁸⁵
Hugorosenone	<i>An. gambiae</i>	<i>Hugonia castaneifolia</i>	LC ₅₀ values of 0.3028 mg/ml	Baraza <i>et al</i> (2008) ⁸⁶
Azadirachtin	<i>An. stephensi</i>	<i>Azadirachta indica</i>	EC ₅₀ values of 0.014, 0.021, 0.028 and 0.034 ppm against first, second, third and fourth instar larvae respectively	Senthil Nathan <i>et al</i> (2005) ⁸⁷
Dioncophylline-A	<i>An. stephensi</i>	<i>Triphyophyllum peltatum</i>	LD ₅₀ values of 0.5, 1.0 and 2.0 mg/L concentrations at 3.33, 2.66 and 1.92 h	Francois <i>et al</i> (1996) ⁸⁸
N-methyl-6β-(decal', 3',5'-trienyl)-3-β-methoxy-2-β-methylpiperidine	<i>Ae. aegyptii</i>	<i>Microcos paniculata</i>	LC ₅₀ value of 2.1 ppm	Bandara <i>et al</i> (2000) ⁸⁹
Stemocurtisine, stemocurtisinol and oxyprotostemonine	<i>An. minimus</i>	<i>Stemona curtisii</i>	LC ₅₀ values of 18, 39 and 4 ppm, respectively	Mungkornasawakul <i>et al</i> (2009) ⁹⁰
Plumbagin	<i>An. gambiae</i>	<i>Plumbago zeylanica</i>	LC ₅₀ value of 1.9 µg/ml	Maniafu <i>et al</i> (2009) ³⁶
Pachyrrhizine	<i>An. gambiae</i>	<i>Neorautanenia mitis</i>	LC ₅₀ value 0.007 mg/ml	Joseph <i>et al</i> (2004) ⁹¹
Marmesin	<i>An. gambiae</i>	<i>Aegle marmelos</i>	LC ₅₀ and LC ₉₀ values of 0.082 and 0.152 mg/l	Joseph <i>et al</i> (2004) ⁹¹
Neoduline, 4-methoxyneoduline, and nepseudin	<i>An. gambiae</i>	<i>Neorautanenia mitis</i>	LD ₅₀ values 0.005, 0.011 and 0.003 mg/ml	Breytenbach & Rall (1980) ⁹²
Methyl-p-hydroxybenzoate	<i>Cx. quinquefasciatus</i> and <i>Ae. aegyptii</i>	<i>Vitex trifolia</i>	LC ₅₀ values of 5.77 and 4.74 ppm, respectively	Kannathasan <i>et al</i> (2011) ⁹³
β-sitosterol	<i>Ae. aegyptii</i> , <i>An. stephensi</i> and <i>Cx. quinquefasciatus</i>	<i>Abutilon indicum</i>	LC ₅₀ value of 11.49, 3.58 and 26.67 ppm, respectively	Rahuman <i>et al</i> (2008) ⁹⁴
Piperonaline	<i>Ae. aegyptii</i> and <i>Cx. pipiens</i>	<i>Piper longum</i>	LC ₅₀ values of 0.25 and 0.21 mg/l, respectively	Lee (2000) ⁹⁵

solvent, combination of solvents or serial extraction by different solvents according to their polarity has also been tried and good larvicidal potentiality found as a result⁹⁶.

Nature of active ingredients responsible for larval toxicity

The plant world comprises a rich untapped pool of phytochemicals that may be widely used in place of synthetic insecticides in mosquito control programme. Kishore *et al*⁹⁷ reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant derived secondary materials, such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpan and lignans. They also documented the isolation of several bioactive toxic principles from various plants and reported their toxicity against different mosquito species (Table II).

Mode of action of phytochemicals in target insect body

Generally the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components⁹⁸. This in turn, affects insect physiology in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system (such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway)⁹⁸. Rattan⁹⁸ reviewed the mechanism of action of plant secondary metabolites on insect body and documented several physiological disruptions, such as inhibition of acetylcholinesterase (by essential oils), GABA-gated chloride channel (by thymol), sodium and potassium ion exchange disruption (by pyrethrin) and inhibition of cellular respiration (by rotenone). Such disruption also includes the blockage of calcium channels (by ryanodine), of nerve cell membrane action (by sabadilla), of octopamine receptors (thymol), hormonal balance disruption, mitotic poisoning (by azadirachtin), disruption of the molecular events of

morphogenesis and alteration in the behaviour and memory of cholinergic system (by essential oil), *etc.* Of these, the most important activity is the inhibition of acetylcholinesterase activity (AChE) as it is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway; AChE has been observed to be organophosphorus and carbamate resistant, and it is well-known that the alteration in AChE is one of the main resistance mechanisms in insect pests⁹⁹.

Scope for future research: isolation of toxic larvicidal active ingredients

Several studies have documented the efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito larvae. But only a few have been commercially produced and extensively used in vector control programmes. The main reasons behind the failure in laboratory to land movements of bioactive toxic phytochemicals are poor characterization and inefficiency in determining the structure of active toxic ingredients responsible for larvicidal activity. For the production of a green biopesticide, the following steps can be recommended during any research design with phytochemicals: (i) Screening of floral biodiversity in search of crude plant extracts having mosquito larvicidal potentiality; (ii) Preparation of plant solvent extracts starting from non-polar to polar chemicals and determination of the most effective solvent extract; (iii) Evaporation of the liquid solvent to obtain solid residue and determination of the lethal concentration (LC_{50}/LC_{100} values); (iv) Phytochemical analysis of the solid residue and application of column chromatography and thin layer chromatography to purify and isolate toxic phytochemical with larvicidal potentiality; (v) Determination of the structure of active principle by infra red (IR) spectroscopic, nuclear magnetic resonance (NMR) and gas chromatography and mass spectroscopy (GCMS) analysis; (vi) Study of the effect of active ingredient on non target organisms; and (vii) Field evaluation of the active principle before its recommendation in vector control programme and commercial production.

Conclusion

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friendly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available

in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. According to Bowers *et al*¹⁰⁰, the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system. The ethno-pharmacological approaches used in the search of new bioactive toxins from plants appear to be predictive compared to the random screening approach. The recently developed new isolation techniques and chemical characterization through different types of spectroscopy and chromatography together with new pharmacological testing have led to an interest in plants as the source of new larvicidal compounds. Synergistic approaches such as application of mosquito predators with botanical blends and microbial pesticides will provide a better effect in reducing the vector population and the magnitude of epidemiology.

Acknowledgment

Authors thank Shri Anindya Sen (Department of English, Bankura Christian College) for critically examine the manuscript. The financial support provided by University Grants Commission to Dr Anupam Ghosh is also acknowledged.

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