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Preliminary evaluation of mosquito larvicidal efficacy of plant extracts

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Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance[1], undesirable effects on non-target organisms and fostered environmental and human health concern[2], which initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals[3] and they may be an alternative source of mosquito control agents.

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Essential oils of leaf and bark of *Cryptomeria japonica* demonstrated high larvicidal activity against *Aedes aegypti* (Diptera: Culicidae) larvae[4]. Insecticidal activity of plant essential oils has been well-described by Isman[5]. Azadiractin, the active ingredient of neem has long been recognised for its mosquito larvicidal capability. The extracts of *Murraya koenigii, Coriandrum sativam, Ferula asafetida* and *Trigonella foenum graceum* were found to be effective and showed encouraging results against *Ae. aegypti*[6] and *Culex* (Diptera: Culicidae) mosquito larvae[7]. It is also reported that many compounds with insecticidal potential have been isolated from the genus *Piper*—Pipercide, isolated from *Piper negrum* (black piper) has been found to be just as active against adjuki bean weevils as the pyrethroides[8]. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities observed by many researchers[9–11]. However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vectors of public health importance.

Northeastern region of India is considered as a major biodiversity hot spot. The eastern Himalayas range, which extends all through the northern border of Assam, is a rich treasure house of many promising medicinal and aromatic plants. In the present communication, an attempt has been made to evaluate the mosquito larvicidal efficacy of methanol and ethanol extracts of different parts of five indigenous plants against *Ae. albopictus* (Diptera: Culicidae) and *Culex quinquefasciatus* larvae in laboratory conditions.

Plant materials were collected from the foothill forests of Sonitpur district, Assam bordering Arunachal Pradesh during April and May 2005. They were segregated as leaf, stem, bark, root and fruit/pericarp and air-dried in a shady place. Dried materials were ground in a table model grinder. The ground plant materials were dipped in solvents (methanol and ethanol) in tightly capped jars separately for 48 h. The solvents along with extracts were drained out, filtered and semisolid extracts were obtained in vacuum using rotary evaporator. The semisolid extracts were lyophilised to obtain solid extracts. Stock solutions of desired concentration were prepared in distilled water using 1 ppm teepol as emulsifying agent and subsequent dilutions were made as per requirement. Larvicidal bioassay was carried out as per standard WHO techniques in 500 ml glass beakers containing 250 ml of water and 25 numbers of late III or early IV instar mosquito larvae for various concentrations. Three different concentrations of each extract were tried out at a time with six replicates. One control was kept with each set of experiment and mortality was recorded after 24 h. Five sets of experiments were conducted for each extract. Tests were carried out under controlled laboratory conditions (temperature 27 ± 2^oC) against laboratory reared *Ae. albopictus* and *Cx. quinquefasciatus* (Diptera: Culicidae) larvae. Values obtained were subjected to log probit regression analysis to obtain LC_{50} and LC_{90} values with 95% confidence limit[12].

The results showed that the larvicidal activity of methanol and ethanol extracts of five aromatic plant species against *Ae. albopictus* and *Cx. quinquefasciatus* larvae varied according to plant species (Tables 1 & 2). Methanol extract of *Aristolochia saccata* roots was found to be the most effective against *Ae. albopictus* larvae followed by ethanol extracts of *A. saccata, Annona squamosa* leaf and methanol extract of *A. squamosa* leaf respectively. LC_{90} values of methanol extract of fruit/pericarp of *Gymnopetelum cochinchinensis*, bark of *Caesalpinea* species and ethanol extract of stem of *Piper* species were obtained at <200 ppm but methanol extract of seeds of *G. cochinchinensis* and stem of *Piper* species gave at <358 ppm against *Ae. albopictus* larvae (Table 1).

Ethanol extract of leaf of *A. squamosa* was found to have the most promising larvicidal activity against *Cx. quinquefasciatus* larvae. Methanol and ethanol extracts of *A. saccata* (root), methanol extract of *A. squamosa* (leaf) showed LC_{90} values at <100 ppm while methanol extract of *G. cochinchinensis* (fruit/pericarp), methanol and ethanol extract of *Piper* species showed at <200 ppm and methanol extract of *G. cochinchinensis* (seed) showed at >302 ppm against *Cx. quinquefasciatus* larvae (Table 2).

Long before the advent of synthetic insecticides, plants and their derivatives were used to kill pest of agriculture, veterinary and public health. Sosan *et al*[13] reported larvicidal activities of essential oils of *Ocimum gratissium, Cymbopogon citrus* and *Ageratum conyzoides* against *Ae. aegypti* and achieved 100% mortality at 120, 200 and 300 ppm concentrations respectively. Similarly, it was reported that the essential oil of *Ipomoea cairica* Linn. possesses remark-

Table 1. Larvicidal efficacy of plant extracts against *Ae. albopictus* larvae

Name of plant	Part used	Solvent used	LC_{50}	LC_{90}	Regression equation
Aristolochia saccata	Root	Methanol	14.52	42.68	Y = 2.5683 *X+2.0164
-do-	Root	Ethanol	17.30	58.51	Y = 2.3633 *X+2.0721
Annona squamosa	Leaf	Methanol	20.26	86.59	Y = 1.9392 *X+2.4637
-do-	Leaf	Ethanol	20.70	76.73	Y = 2.1991 *X+2.1020
Gymnopetelum cochinchinensis	Fruit/Pericarp	Methanol	50.67	155.12	Y = 2.5821 *X+0.5927
-do-	Seed	Methanol	100.42	312.45	Y = 2.3014 *X+0.3481
Caesalpinea species	Bark	Methanol	53.66	169.41	Y = 2.3429*X+0.8638
Piper species	Stem	Methanol	144.22	357.32	Y = 3.1826 *X–1.9688
-do-	Stem	Ethanol	76.35	180.42	Y = 3.2525 *X–1.1333

Table 2. Larvicidal efficacy of plant extracts against *Cx. quinquefasciatus* larvae

Name of plant	Part used	Solvent used	LC_{50}	LC_{90}	Regression equation
Aristolochia saccata	Root	Methanol	31.91	81.06	Y = 3.3086 *X-0.0062
-do-	Root	Ethanol	19.83	60.44	Y = 2.5791 *X-1.6605
Annona squamosa	Leaf	Methanol	17.70	64.29	Y = 2.1180 *X+2.3457
-do-	Leaf	Ethanol	6.96	31.80	Y = 1.9441 *X+3.3592
Gymnopetelum cochinchinensis	Fruit/pericarp	Methanol	57.4	108.3	Y = 4.1627 *X+2.3501
-do-	Seed	Methanol	199.0	301.6	Y = 1.7586 *X+11.0557
Caesalpinea species	Bark	Methanol	42.27	207.13	Y = 1.7586 *X+2.0573
Piper species	Stem	Methanol	70.10	113.90	Y = 5.669 *X+5.4992
-do-	Stem	Ethanol	57.4	108.3	Y = 4.1627 *X+2.3501

able larvicidal properties as it could produce 100% mortality in the larvae of *Cx. tritaeniorhynchus, Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* mosquitoes at concentrations ranging from 100 to 170 ppm[14]. Dwivedi & Kawasara[15] found acetone extract of *Lantana camara* to be most effective against *Cx. quinquefasciatus* larvae at the dose of 1 ml/100 ml. Latha *et al*[16] reported *Piper longum* and *Zingiber wightianum* extracts at 80 mg/l causing complete mortality in *Cx. quinquefasciatus* and 60 mg/l for *Cx. sitiens*. In the present investigation LC_{90} values of methanol and ethanol extracts of roots of *A. saccata*, leaf of *A. squamosa* and fruits/pericarp of *G. cochinchinensis* against *Ae. albopictus* and *Cx. quinquefasciatus* larvae ranged between 31.80 and 155 ppm. Studies with essential oil of *Ocimum americans* and *O. gratissium* showed LC_{50} at 67 and 60 ppm respectively against *Ae. aegypti* larvae[17]. In contrast, in the present study methanol and ethanol extracts of roots of *A. saccata*, leaf of *A. squamosa* and fruits/pericarp of *G. cochinchinensis* against *Ae. albopictus* and *Cx. quinquefasciatus* larvae showed LC_{50} values between 6.96 and 57.4 ppm. Larvicidal activities of the plant extracts vary according to the plant species, the parts of the plant, the geographical location where the plants were grown and the application method.

Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals for control of insect vectors.

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