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Rajasingh Raveen
Department of Zoology,
Madras Christian College,
Chennai, Tamil Nadu, India

Fauzia Ahmed
1) Department of Zoology,
Madras Christian College,
Chennai, Tamil Nadu, India
2) Department of Zoology, JBAS College for Women,
Chennai, Tamil Nadu, India

Muniyasamy Pandeeswari
1) Department of Zoology,
Madras Christian College,
Chennai, Tamil Nadu, India
2) Department of Zoology,
S.I.V.E.T. College,
Chennai, Tamil Nadu, India

Daniel Reegan
Department of Zoology,
Madras Christian College,
Chennai, Tamil Nadu, India

Samuel Tennyson
Department of Zoology,
Madras Christian College,
Chennai, Tamil Nadu, India

Subramanian Arivoli
Department of Zoology,
Thiruvalluvar University,
Vellore, Tamil Nadu, India

Manickkam Jayakumar
Department of Zoology,
University of Madras,
Chennai, Tamil Nadu, India

Correspondence
Dr. Rajasingh Raveen
Associate Professor,
Department of Zoology,
Madras Christian College,
Chennai, Tamil Nadu, India
raveenraja2002@gmail.com

Laboratory evaluation of a few plant extracts for their ovicidal, larvicidal and pupicidal activity against medically important human dengue, chikungunya and Zika virus vector, *Aedes aegypti* Linnaeus 1762 (Diptera: Culicidae)

Rajasingh Raveen, Fauzia Ahmed, Muniyasamy Pandeeswari, Daniel Reegan, Samuel Tennyson, Subramanian Arivoli and Manickkam Jayakumar

Abstract
Vector control is by far the most successful method for reducing incidences of mosquito-borne diseases. Plant products have been tested as insecticides against mosquitoes as they are nontoxic to mammals and are promising candidates to replace conventional insecticides. In the present study, the bioefficacy of crude solvent leaf extracts of *Alternanthera sessilis* (Amaranthaceae), *Amaranthus dubius* (Amaranthaceae), *Sesbania grandiflora* (Fabaceae) and *Solanum nigrum* (Solanaceae) were tested on the immature stages of the dengue, chikungunya, yellow fever and Zika virus vector, *Aedes aegypti*. The eggs, larvae and pupae of *Aedes aegypti* were exposed to different concentrations *viz*., 62.5, 125, 250 and 500mg/L. After 24 hours of exposure, the larval and pupal per cent mortality was assessed and their LC_{50} and LC_{90} values calculated. However, per cent egg mortality was calculated after 72 hours of exposure. Among the different crude solvent extracts of the plants tested, the butanol extract of *Alternanthera sessilis* caused highest egg mortality rate of 86% and the ethanol extract of *Solanum nigrum* exhibited highest larvicidal and pupicidal activity and LC_{50} and LC_{90} values were 34.12, 88.11 and 44.15, 111.70mg/L respectively. The successful results of preliminary studies on mosquitocidal potential of plant extracts encourage further effort to investigate the bioactive compounds in those extracts that might possess good immature mosquitocidal properties when isolated in pure form.

Keywords: Crude solvent leaf extracts, ovicidal, larvicidal, pupicidal, *Aedes aegypti*

1. Introduction
Mosquitoes are the major vectors of many pathogens which cause diseases like dengue, chikungunya, yellow fever, Zika virus fever, malaria, filariasis and Japanese encephalitis [1, 2]. Mosquito-borne diseases continue to have a devastating impact on human beings for the past several decades [3, 4]. Mosquito-borne diseases cause high levels of economic impact throughout the world [5-7] and infect over 700,000,000 people every year globally and 40,000,000 of the Indian population [8]. *Aedes aegypti* Linnaeus (Diptera: Culicidae) is generally known as vector of arboviruses as it is responsible for transmission of dengue, chikungunya and Zika virus fever [1, 2]. *Aedes aegypti* is endemic to Southeast Asia, the Pacific island area, Africa and Americas [9]. A study estimates that 3,900 million people, in 128 countries, are at risk of infection with dengue viruses alone [10].
Vector mosquito control or management is important in order to improve public health. Mosquito control, in view of their medical importance, assumes global importance. Vector control is by far the most successful method for reducing incidences of mosquito-borne diseases [11]. Chemical pesticides are proved to be effective in controlling the mosquito population. In the context of ever increasing trend to use more powerful synthetic insecticides to achieve immediate results in the control of mosquitoes, an alarming increase of physiological resistance in the vectors and its increased toxicity to non-target organisms are noteworthy [12].

Plant extracts are believed to be a good alternative to chemical insecticides. Many plant natural products have been tested as insecticides against mosquitoes [13-16] as they are nontoxic to mammals and are promising candidates to replace conventional insecticides [17-20]. Members of the plant families, Asteraceae, Cladophoraceae, Lamiaceae, Miliaceae, Oocystaceae, Rutaceae and Solanaceae have various types of larvicidal, adulticidal or repellent activities against different species of mosquitoes [18]. A brief delve into the literature reveals many investigations made towards the biological screening of botanical extracts and the activity of many plant derived components against mosquitoes [8,18,21-31] and in the current scenario, several researchers are searching locally available plant materials in order to find out eco-friendly products to manage different mosquito species [32-40]. Therefore, in the expedition towards natural plant products for mosquito control, the present study was aimed to analyze the biocidal activity of *Alternanthera sessilis*, *Amaranthus dubius*, *Sesbania grandiflora* and *Solanum nigrum* crude solvent leaf extracts on the immature stages of *Aedes aegypti*.

2. Materials and methods

2.1. Plant collection and preparation of phytoextracts

Mature and healthy leaves of *Alternanthera sessilis* (Amaranthaceae), *Amaranthus dubius* (Amaranthaceae), *Sesbania grandiflora* (Fabaceae) and *Solanum nigrum* (Solanaceae) were collected from Chennai, Tamil Nadu, India. Taxonomical identity of the plant was confirmed at Department of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai, India. The leaves of each plant were then brought to the laboratory, washed in dechlorinated water, shade dried for five days and powdered with the aid of an electric blender. The powdered leaves (1Kg) of each plant were extracted with different solvents (3L) each *viz*., hexane, butanol, propanol and ethanol in a soxhlet apparatus [41] with minor modifications. The crude solvent leaf extract of each plant was stored in air tight amber coloured bottles.

2.2. Test mosquitoes

Cyclic generations of the *Aedes aegypti* vector mosquitoes free of exposure to insecticides were maintained in mosquito cages (2'x2'x2') in an insectary with a mean room temperature of 27 ±2 °C and a relative humidity of 70-80%. The adult mosquitoes were fed on ten per cent glucose solution in water. The eggs laid in ovitraps placed inside the mosquito cages were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside another mosquito cage (2'x2'x2') for adult emergence.

2.3. Ovicidal bioassay

Ovicidal bioassay was carried out as per the guidelines of Reegan *et al*. [15] with minor modifications. Twenty five numbers of *Aedes aegypti* eggs were introduced into each test container. The extracts were tested at different concentrations *viz*., 62.5, 125, 250 and 500mg/L. Acetone in distilled water was used as treated control, whereas distilled water served as untreated control. Unhatched eggs in each concentration were registered after 72 hours of exposure period. A total of five replicates per trial for each concentration were conducted. Mortality was converted into mean per cent egg mortality using the following formula and was expressed as Egg Mortality Rate (EMR).

$$EMR = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs exposed}} \times 100$$

2.4. Larvicidal and pupicidal bioassay

Larvicidal bioassay was carried out as per the guidelines of World Health Organization [42] with minor modifications. Larvicidal activity at test concentrations of 62.5, 125, 250 and 500mg/L of each crude leaf extract was assessed. The required test concentrations and quantity of test solution was prepared by serially diluting one per cent stock solution of the crude extract. Twenty early third instar larvae from laboratory colonized mosquitoes of F_1 generation were introduced into glass beakers (250mL) containing 200mL of distilled water and test concentration. Mortality was observed 24 hours after treatment. Untreated control (distilled water only), treated control (acetone in distilled water) and positive control (Temephos at concentration of 2.5, 5.0, 7.5 and 10.0mg/L) were maintained separately and run simultaneously. A total of five replicates per trial for each concentration were carried out. The per cent larval mortality was calculated using the formula (1) and corrections for control mortality (5 – 20%) when necessary was done using formula (2) of Abbott's [43]. Likewise, the same methodology was adopted for pupicidal bioassay. Statistical analysis of all mortality data of larvicidal and pupicidal bioassay were subjected to probit analysis [44]. The differences were considered as significant at $P \leq 0.05$ level.

Per cent larval mortality (1):

$$\frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/pupae introduced}} \text{ x } 100$$

Corrected percentage of control mortality (2):

$$\frac{1 - n \text{ in T after treatment}}{n \text{ in C after treatment}} \text{ x } 100$$

Where, *n* is the number of larvae/ pupae,
T: treated and C: control.

3. Results

The crude solvent leaf extracts of the plants tested against the immature stages of *Aedes aegypti* exhibited varied mortality. No mortality of *Aedes aegypti* immatures was observed in treated and untreated control. Maximum EMR of 86, 74, 70 and 60% was exhibited by the butanol extract of *Alternanthera sessilis*, *Amaranthus dubius*, *Solanum nigrum* and *Sesbania grandiflora* respectively (Figure 1). Temephos served as positive control and indicated 48% EMR. Larval mortality varied between extracts of different plants tested. The ethanol extract was found to exhibit the highest larvicidal activity and the order of LC_{50} values were 34.12, 51.16, 91.17 and 130.37mg/L in *Solanum nigrum*, *Sesbania grandiflora*, *Alternanthera sessilis* and *Amaranthus dubius* respectively (Table 1; Figure 2). Likewise for pupal mortality, the same trend followed where ethanol extract was effective in all the plant extracts tested except in *Alternanthera sessilis* where it

was replaced by the butanol extract. The respective LC_{50} values were 44.15, 57.79, 63.29 and 140.70mg/L (Table 2; Figure 3). Overall assessment indicated the butanol extract of *Alternanthera sessilis* to exhibit the highest ovicidal activity and the ethanol extract of *Solanum nigrum* for larvicidal and pupicidal activity against *Aedes aegypti*.

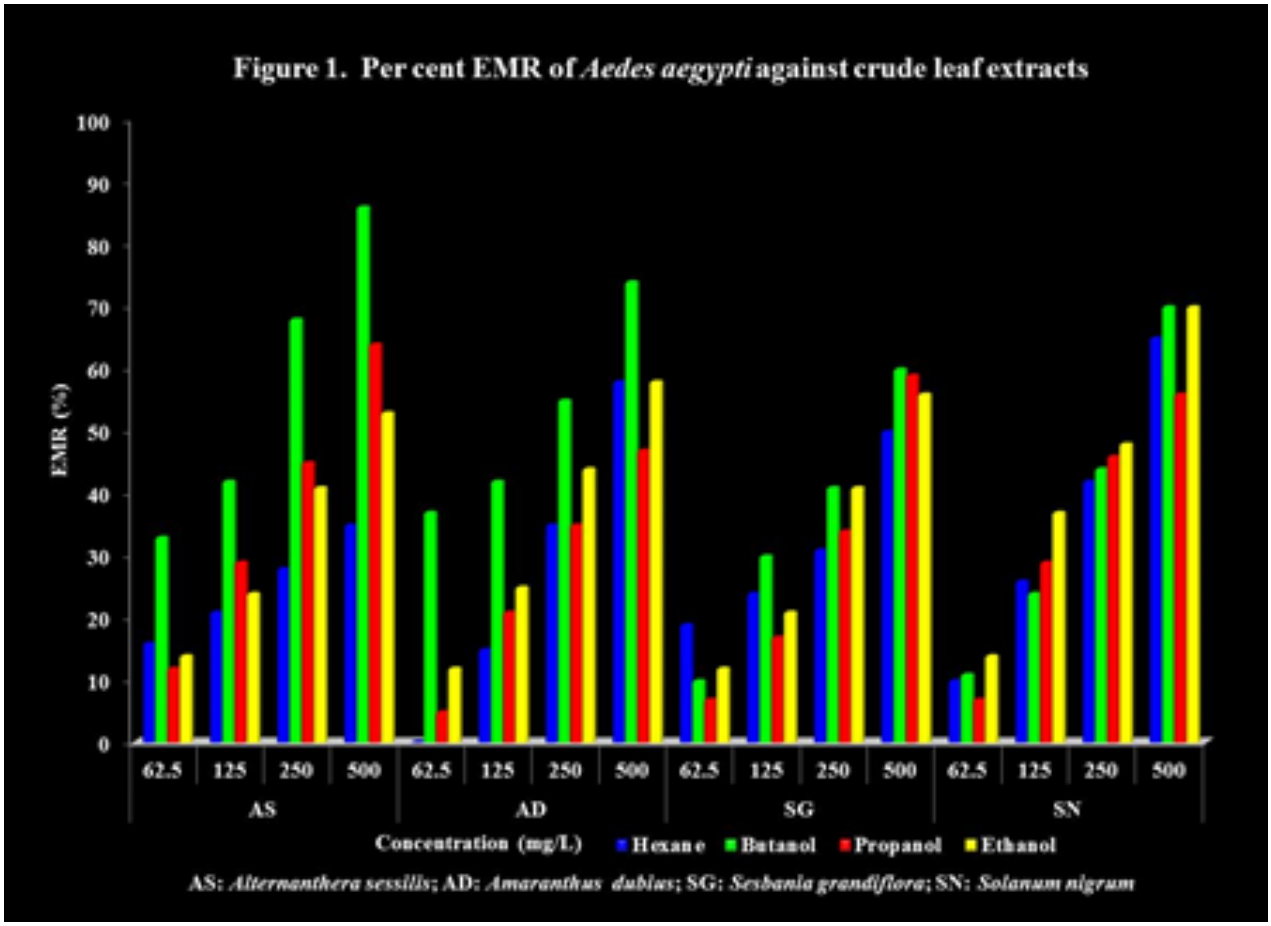


Table 1: Larvicidal activity of crude leaf extracts against *Aedes aegypti*

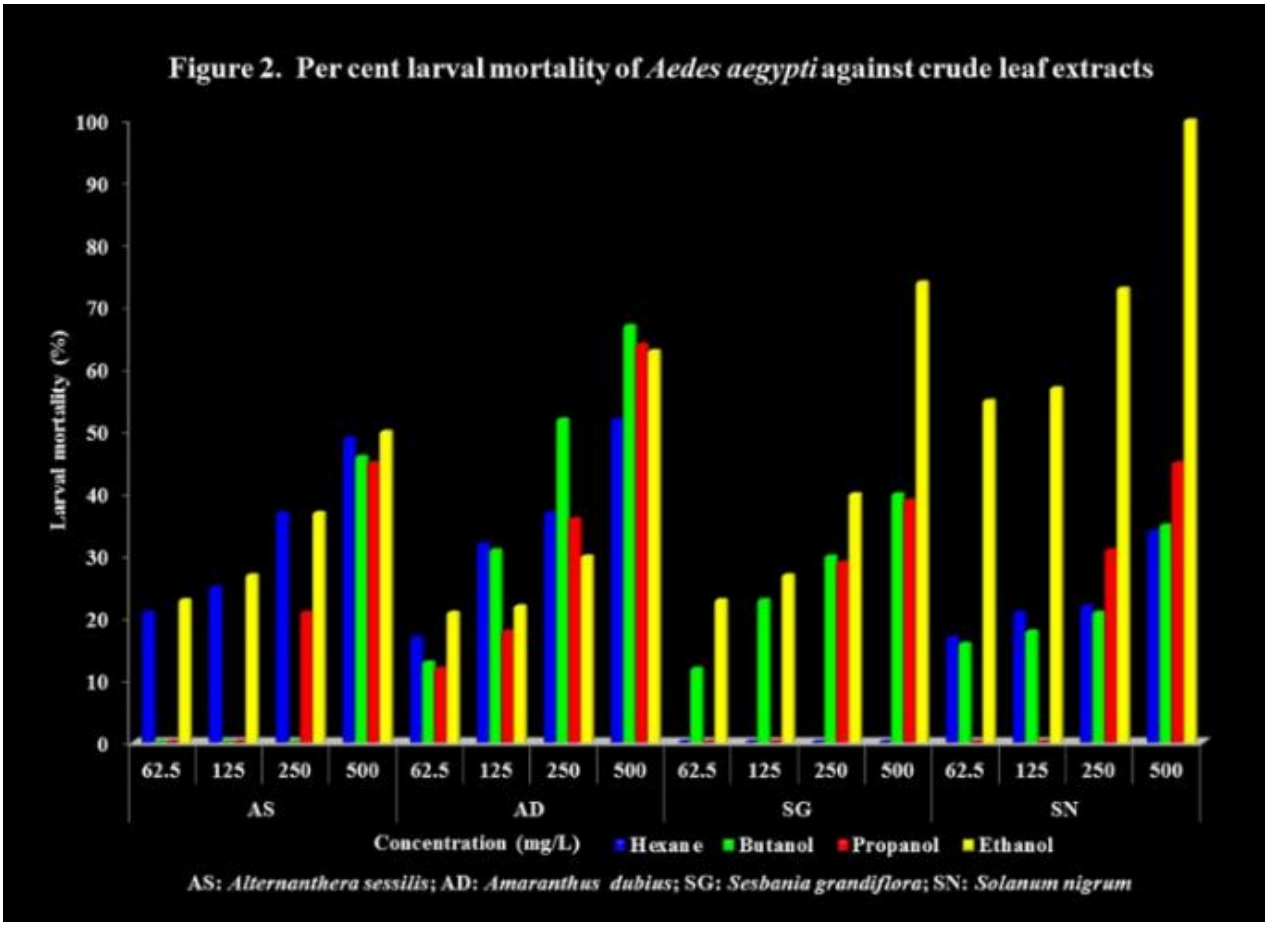
Solvents	LC_{50} (mg/L)	95% confidence limit		LC_{90} (mg/L)	95% confidence limit		Slope ±SE	Intercept ±SE	χ^2
		LL	UL		LL	UL			
Alternanthera sessilis									
Hexane	163.81	127.38	209.28	394.69	291.09	688.14	3.3 ±0.6	-2.4 ±1.3	0.3*
Butanol	54.79	22.18	74.45	130.79	97.77	279.71	3.3 ±1.0	-0.8 ±2.0	0.5*
Propanol	75.94	48.12	98.89	187.87	139.96	352.81	3.2 ±0.7	-1.1 ±1.6	0.1*
Ethanol	91.17	66.08	115.39	206.71	156.63	359.88	3.6 ±0.7	-2.0 ±1.6	0.2*
Amaranthus dubius									
Hexane	161.21	6.86	397.56	408.93	207.92	783.11	3.1 ±0.7	-1.9 ±1.5	4.7*
Butanol	320.94	187.23	516.25	891.73	1158.12	1727.79	2.8 ±0.7	-2.2 ±1.7	3.6*
Propanol	249.21	186.79	358.40	790.65	498.94	2163.27	2.5 ±0.5	-1.1 ±1.2	1.9*
Ethanol	130.37	98.81	166.50	321.88	237.75	564.95	3.2 ±0.6	-1.9 ±1.3	1.5*
Sesbania grandiflora									
Hexane	208.30	181.12	451.90	479.32	294.91	780.22	3.5 ±1.0	-3.2 ±2.4	7.1*
Butanol	186.90	77.13	291.87	447.55	317.32	593.42	3.3 ±0.8	-2.6 ±1.9	7.6*
Propanol	228.78	112.56	574.73	539.79	376.26	831.80	2.3 ±0.4	-1.4 ±1.2	8.8*
Ethanol	51.16	14.29	67.18	100.87	78.15	260.10	4.3 ±1.6	-2.4 ±3.1	0.1*
Solanum nigrum									
Hexane	269.25	242.91	300.54	623.51	526.40	782.18	3.51 ±0.3	-3.5 ±0.7	2.4*
Butanol	328.03	289.58	379.53	911.4	719.49	1273.88	2.8 ±0.2	-2.2 ±0.6	5.6*
Propanol	94.33	68.76	119.49	216.40	163.52	376.19	3.5 ±0.7	-2.0 ±1.5	0.7*
Ethanol	34.12	13.31	56.58	88.11	45.69	805.80	3.1 ±1.5	0.2 ±2.8	0.1*
Temephos	1.10	0.21	1.89	2.31	1.38	3.18	3.9 ±0.4	5.1 ±0.1	5.8*

LC_{50}: lethal concentration that kills 50% of the exposed larvae; LC_{90}: lethal concentration that kills 90% of the exposed larvae; LL: lower limit; UL: upper limit; *$P \leq 0.05$ level of significance of chi-square values

Table 2: Pupicidal activity of crude leaf extracts against *Aedes aegypti*

Solvents	LC_{50} (mg/L)	95% confidence limit		LC_{90} (mg/L)	95% confidence limit		Slope ±SE	Intercept ±SE	χ^2
		LL	UL		LL	UL			
Alternanthera sessilis									
Hexane	185.61	145.45	237.81	442.27	325.17	777.14	3.3 ±0.6	-2.7 ±1.4	3.7*
Butanol	63.29	31.33	86.68	175.54	127.78	361.59	2.8 ±0.7	-0.2 ±1.5	0.1*
Propanol	79.83	58.43	99.08	158.35	123.47	272.08	4.3 ±1.0	-3.1 ±2.0	0.7*
Ethanol	105.74	80.48	132.68	232.38	177.11	389.45	3.7 ±0.7	-2.5 ±1.5	1.7*
Amaranthus dubius									
Hexane	263.95	76.26	359.07	654.99	324.76	875.27	3.2 ± 0.7	-2.8 ±1.7	3.4*
Butanol	377.88	147.23	518.74	1001.63	969.34	1412.77	3.0 ± 0.9	-2.8 ±2.1	3.1*
Propanol	288.98	220.49	415.40	823.47	531.86	2135.52	2.8 ± 0.5	-1.9 ±1.4	1.0*
Ethanol	140.70	109.69	177.51	320.50	241.38	535.20	3.5 ± 0.6	-2.7 ±1.4	1.4*
Sesbania grandiflora									
Hexane	568.49	450.63	807.42	2469.77	1502.20	5656.27	2.0 ±0.2	-0.5 ±0.6	2.3*
Butanol	266.49	147.33	413.56	660.90	437.38	914.66	3.2 ±0.9	-2.8 ±2.3	3.7*
Propanol	306.61	159.66	739.45	926.90	427.35	615.75	2.6 ±0.5	-1.6 ±1.2	8.3*
Ethanol	57.79	23.69	82.17	174.04	124.44	380.27	2.6 ±0.7	0.2 ±1.5	0.1*
Solanum nigrum									
Hexane	524.66	427.11	707.13	2030.26	1314.33	4102.59	2.1 ±0.2	-0.9 ±0.6	1.5*
Butanol	401.39	343.53	490.73	1321.01	959.12	2136.12	2.4 ±0.2	-1.4 ±0.6	4.2*
Propanol	102.29	77.55	128.28	223.33	170.49	375.07	3.7 ±0.7	-2.5 ±1.6	0.9*
Ethanol	44.15	6.42	64.91	111.70	80.26	287.09	3.1 ±1.1	-0.2 ±2.3	0.3*
Temephos	1.50	0.42	2.76	2.84	1.55	3.99	3.7 ±0.1	2.2 ±0.2	4.1*

LC_{50}: lethal concentration that kills 50% of the exposed pupae; LC_{90}: lethal concentration that kills 90% of the exposed pupae; LL: lower limit; UL: upper limit; *$P \leq 0.05$ level of significance of chi-square values



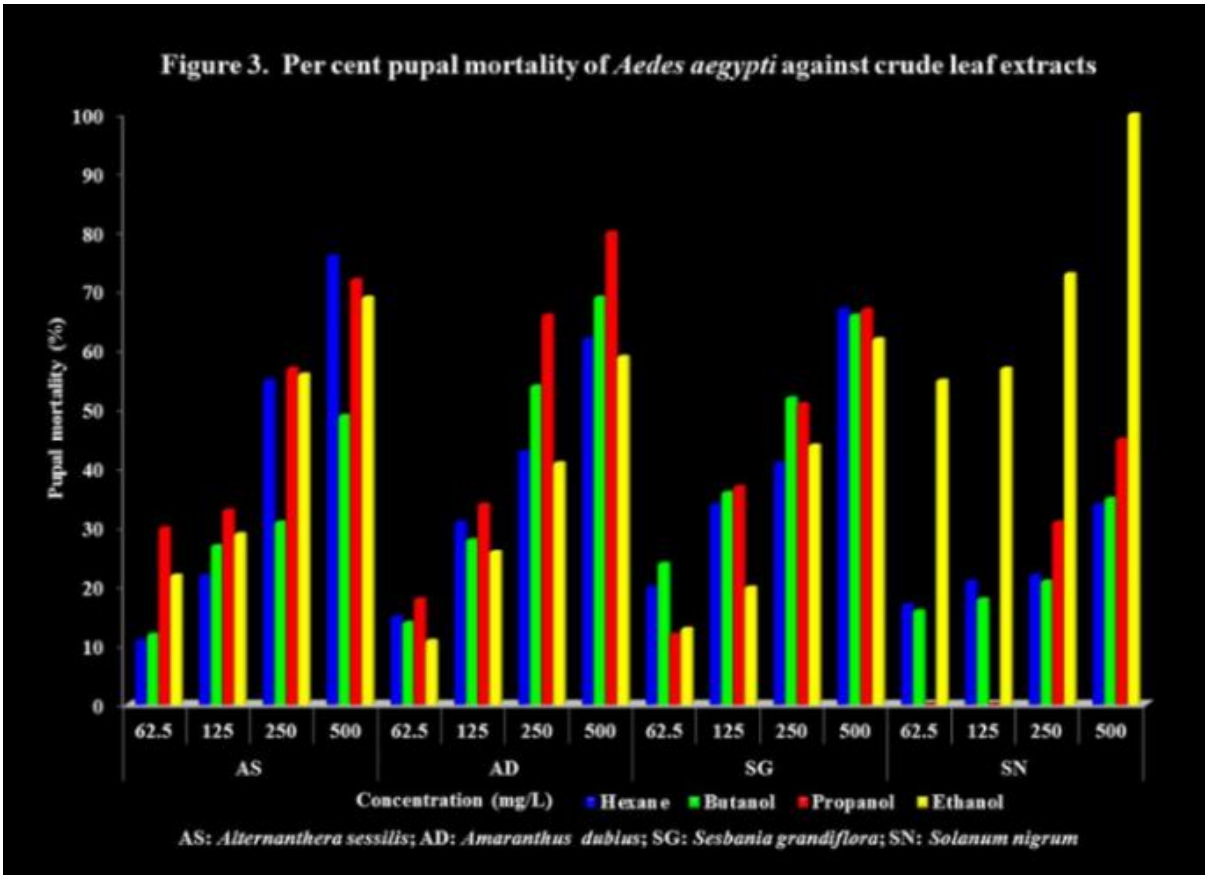


Table 3: List of ethanolic plant extracts reported for mosquito larvicidal activity

Plant species	Part	LC_{50}	Mosquito species	Work cited
Cannabis sativa	Leaf	1000mg/L	*Anopheles stephensi*	Jalees *et al.* [48]
Tagetes minuta	Aerial parts	1.0mg/L	*Aedes fluviatilis*	Macedo *et al.* [49]
Eclipta prostrata		3.3mg/L		
Azadirachta indica	Leaf	0.45ppm	*Culex fatigans*	Azmi *et al.* [50]
Piper nigrum	Seed	26.0mg/L	*Culex pipiens*	Moawed *et al.* [51]
Calotropis procera	Root	28.0mg/L	*Anopheles labranchiae*	Markouk *et al.* [52]
Rhizophora mucronata	Bark	157.4ppm	*Aedes aegypti*	Kabaru and Gichia [53]
	Pith	168.3ppm		
	Stem wood	1003.4ppm		
Apium graveolens	Seed	81.0mg/L	*Aedes aegypti*	Choochote *et al.* [54]
	Fruit	2.23ppm		Chaithong *et al.* [55]
Piper longum	Fruit	2.23ppm	*Aedes aegypti*	Chaithong *et al.* [55]
Piper ribesoides	Wood	8.13ppm		
Piper sarmentosum	Whole plant	4.06ppm		
Citrus reticulata	Seed	2267.71ppm	*Aedes aegypti*	Sumroiphon *et al.* [56]
		2639.27ppm	*Culex quinquefasciatus*	
Annona crassiflora	Root wood	0.71mg/L	*Aedes aegypti*	Omena *et al.* [57]
	Root bark	8.94mg/L		
	Stem	16.1mg/L		
Annona glabra	Seed	0.06µg/mL		
Annona muricata	Root	42.3µg/mL		
Annona squamosa	Root	31.9µg/mL		
	Leaf	169µg/mL		
Deris species	Root	8.54µg/mL		
Erythrina mulungu	Stem bark	67.9µg/mL		
Pterodon polygalaeflorus	Seed	35.79µg/mL		
Cassia obtusifolia	Leaf	52.2mg/L	*Anopheles stephensi*	Rajkumar and Jebanesan [58]
Allium sativum	Bulb	165.70ppm	*Culex quinquefasciatus*	Kalu *et al.* [59]
Azadirachta indica	Leaf	8.32 mg/mL	*Aedes aegypti*	Mgbemena [60]
Ocimum gratissimium		19.50mg/mL		
Citrus citratus		34.67mg/mL		
Millettia pachycarpa	Root bark	98.47ppm	*Aedes aegypti*	Lalchhandama [61]

Exacum pedunculatum	Leaf	121.24ppm	*Anopheles stephensi*	Elangovan *et al.* [62]
Carica papaya	Leaf	5.41%	*Anopheles stephensi*	Kovendan *et al.* [63]
Gliricidia sepium	Leaf	121.79 ppm	*Anopheles stephensi*	Krishnappa *et al.* [64]
Solanum xanthocarpum	Leaf	193.82 ppm	*Culex quinquefasciatus*	Kumar *et al.* [65]
Acalypha alnifolia	Leaf	6.88%	*Anopheles stephensi*	Murugan *et al.* [66]
Biophytum sensitivum	Leaf	633.6481µgmL^{-1}	*Aedes aegypti*	Shivakumar *et al.* [67]
Datura stramonium	Leaf	86.25ppm	*Aedes aegypti*	Swathi *et al.* [68]
		16.07 ppm	*Anopheles stephensi*	
		6.25 ppm	*Culex quinquefasciatus*	
Acalypha indica	Leaf	665.95ppm	*Aedes aegypti*	Vijayakumar *et al.* [69]
		988.42ppm	*Anopheles stephensi*	
		656.4ppm	*Culex quinquefasciatus*	
Solanum nigrum	Stem	90.20 ppm	*Culex quinquefasciatus*	Yoganath *et al.* [70]
Celosia arntea	Leaf	172.31ppm	*Anopheles stephensi*	Dhanasekaran *et al.* [71]
		134.4 ppm	*Aedes aegypti*	
		173.73 ppm	*Culex tritaeniorhynchus*	
Anthocephalus cadamba		109.87 ppm	*Anopheles stephensi*	
		154.09 ppm	*Aedes aegypti*	
		157.68 ppm	*Culex tritaeniorhynchus*	
Gnetum ula		82.86 ppm	*Anopheles stephensi*	
		135.1 ppm	*Aedes aegypti*	
		160.97 ppm	*Culex tritaeniorhynchus*	
Solena amplexicaulis		125.91 ppm	*Anopheles stephensi*	
		153.68 ppm	*Aedes aegypti*	
		109.37 ppm	*Culex tritaeniorhynchus*	
Spermacoce hispida		89.45 ppm	*Anopheles stephensi*	
		177.2 ppm	*Aedes aegypti*	
		99.32 ppm	*Culex tritaeniorhynchus*	
Cyperus rotundus	Rhizome	594.22%	*Aedes aegypti*	Imam *et al.* [72]
Annona reticulata	Leaf	132.636mg/L	*Aedes aegypti*	Govindarajulu *et al.* [73]
Citrullus colocynthis	Seed	30.90 ppm	*Culex quinquefasciatus*	Hamid *et al.* [74]
		39.81 ppm	*Anopheles arabiensis*	
	Fruit pulp	50.11 ppm	*Culex quinquefasciatus*	
		25.12 ppm	*Anopheles arabiensis*	

Table 4: List of ethanolic plant extracts reported for mosquito pupicidal activity

Plant species	Part	LC_{50}	Mosquito species	Work cited
Azadirachta indica	Seed kernel	1.70%	*Aedes aegypti*	Abba *et al.* [75]
Piper nigrum	Seed	3546.1	*Aedes aegypti*	Briones and Garbo [76]
Mimosa pudica	Leaf	2.835ppm	*Anopheles stephensi*	Aarthi and Murugan [77]
Cassia occidentalis	Leaf	92.21%	*Anopheles stephensi*	Abirami and Murugan [78]
Xylopia aethiopica	Fruit	0.40µg/mL	*Anopheles gambiae*	Akinkurolere *et al.* [79]
Myrianthus arboreus	Bark	0.64µg/mL		
Citrus sinensis	Peel	490.84ppm	*Anopheles stephensi*	Murugan *et al.* [80]
		497.41ppm	*Aedes aegypti*	
		530.97ppm	*Culex quinquefasciatus*	
Biophytum sensitivum	Leaf	23.43694µgmL^{-1}	*Aedes aegypti*	Shivakumar *et al.* [67]
Coutarea hexandra	Shell	90725.0µg/mL	*Aedes aegypti*	Candido *et al.* [81]
Cnidoscolus phyllacanthus		715.8µg/mL		
Schinus molle	Leaf	60% mortality (100mg/L)	*Culex quinquefasciatus*	Girmay *et al.* [82]
Terminalia catappa	Leaf	169.8ppm	*Aedes aegypti*	Unnikrishnan [83]
Turbinaria conoides	Seaweed/whole	115.37	*Aedes aegypti*	Valentina *et al.* [84]
		36.82	*Anopheles stephensi*	
		123.3	*Culex quinquefasciatus*	
Cosmos bipinnatus	Leaf	1.14mg/µL	*Culex quinquefasciatus*	Modise and Ashafa [85]
Foeniculum vulgare		1.31mg/µL		
Tagetes minuta		1.11mg/µL		

4. Discussion

Synthetic chemicals have been proved to be effective, but they cause adverse effects on the environment and human health [45]. In this situation, ecofriendly alternatives are important for safer control of mosquitoes. 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 [8]. The search for natural and benign environmental mosquitocides is ongoing worldwide [46, 47]. Natural product literature provides a growing research on plant derived mosquitocidal agents [24].

Plant extracts have been screened and studied for their ovicidal activity against mosquitoes [22]. Ouda *et al.* [86] firstly

investigated the ovicidal potential of botanical products against mosquitoes, showing that doses ranging from 0.1 to 1000ppm of *Atriplex canescens* seed extract possess weak ovicidal properties against *Culex quinquefasciatus* (i.e. egg hatching reduction of 28.8% when tested at 1000ppm). The potential of plant extracts as effective ovicides was studied by Govindarajan and Karuppannan [87] who reported the ovicidal efficacy of benzene, hexane, ethyl acetate, methanol and chloroform leaf extracts of *Eclipta alba* and reported a zero hatchability when eggs were exposed to 300ppm of its methanolic extracts. Rare reports have been documented on the butanol plant extracts for its toxicity on the eggs of mosquitoes. The butanol extract of soapberry plant, *Phytolacca dodecandra* was very toxic to second and third instar larvae of *Aedes aegypti*, *Culex pipiens* and *Anopheles quadrimaculatus*, but not toxic to the eggs and pupae of the above tested mosquito species [88]. However, in the present study, the butanol extracts of different plants tested was found to be toxic to the eggs of *Aedes aegypti*. de Souza *et al*. [13] highlighted that none of the seed ethanolic extract of twenty one Brazilian plants was able to exert 100% mortality against *Aedes aegypti* eggs. Whereas the results of the present study revealed that the ethanol extract exhibited 50-70% ovicidal activity which reasonably corroborates with the results of other reports wherein one hundred per cent egg mortality (zero per cent egg hatchability) was observed in the ethanolic extracts of plants which are as follows. Leaves of *Celosia argentea*, *Anthocephalus cadamba*, *Gnetum ula*, *Solena amplexicaulis* and *Spermacoce hispida* at 250ppm against the eggs of *Anopheles stephensi*, *Aedes aegypti* and egg rafts of *Culex tritaeniorhynchus* except for *Anthocephalus cadamba* at 500ppm [71]; *Exacum pedunculatum* against *Anopheles stephensi* at 200ppm [62]; *Gliciridia sepium* against *Anopheles stephensi* at 100ppm [64]; root bark of *Millettia pachycarpa* at 200ppm against *Aedes aegypti* [61] and *Scutellaria violacea* at 250ppm against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* [89]. However, certain studies reported 50 and <50% egg mortality. Leaf [90] and stem [91] extract of *Argemone mexicana* exhibited only 50% egg mortality against *Aedes aegypti*. Leaf extract of *Andrographis paniculata* against *Anopheles subpictus* produced <40% egg mortality at 46ppm [92]. Kuppusamy *et al*. [92] reported that the ovicidal activity indicated a significant result that the larvae which hatched out of the treated eggs were succumbed to death by an hour or two. In the case of ovicidal activity, exposure to the freshly laid eggs was more effective than that to the older eggs. Singh and Mittal [93] reported that the seed extract of *Solanum nigrum* significantly concentrated the amount of eggs deposited by gravid *Anopheles stephensi* and at the highest dose (0.5%) egg laying was reduced up to 99%.

Maximum EMR rate at a low concentration is an indication of potential ovicidal activity. Ovicidal activity is reported to be influenced by concentration, age of eggs, formulation and mosquito species [94]. Mosquito eggs are soft and white at oviposition but undergo sclerotization during embryogenesis becoming hard and dark. The embryo within the laid egg is protected by a shell which comprises protease-resistant envelope, namely, the roughly homogenous endochorion and the compound exochorion which is made up of an internal lamellar layer and external protruding tubercles [95]. Metabolites of specific amino acids are critical for the formation and maturation of the egg chorion. Ovicidal compounds are able to interrupt embryo development, impair the survival of larva inside the egg or block egg hatching. In the present study, fresh eggs from control showed embryogenesis in progress while impairment of embryo development was detected in treated eggs, reflecting ovicidal activity as reported by Govindarajan *et al*. [96] and Madhiyazhagan *et al*. [97]. The extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion. It is evident from the present study that exposure of *Aedes aegypti* eggs to the leaf extracts of various solvents of the plant extracts tested not only elicited egg mortality but also delayed hatchability to larval stages in particular butanol followed by ethanol extract. Similar kind of observation was noted by Aarthi and Murugan [77], Rajkumar *et al*. [98] and Selvakumar *et al*. [99].

Plant parts containing alkaloids, coumarins, flavonoids, quinines, saponins, steroids and terpenoids [100,101] may be toxic to the immature mosquitoes. Liu *et al*. [102] considered alkaloids among the active molecules to be toxic to mosquito larvae. Alkaloids are nitrogenous compounds that show insecticidal properties at low concentration and the mode of action on insect vectors varies with the structure of their molecules, but many are reported to affect acetylcholinestrase (AChE) or sodium channels as inhibition of acetylcholinesterase activity is responsible for terminating the nerve impulse transmission through synaptic pathway [103]. Alkaloids work by constricting blood vessels and depressing autonomic nervous system activity, thereby contributing to the insecticide's effectiveness in killing the larvae of mosquitoes and disrupting the life cycle of the mosquito [104]. Macedo *et al*. [49] screened ethanolic aerial extracts of eighty three plants belonging to Asteraceae family for larvicidal activity against *Aedes fluviatilis* of which twenty seven caused significant lethality. Details of larvicidal (Table 3) and pupicidal (Table 4) activities of ethanolic plant extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquitoes corroborates with the results of the present study. Ethanolic leaf extracts contain terpenoids, flavonoids, saponins, steroids and tannins that play a role in plant defence against insect pests, and might have been responsible for larval and pupal deaths [105]. There are several studies of the activity of plant extracts on *Aedes aegypti*, mainly examining its larvicidal action. It is found that botanical derivatives possessing mosquito larvicidal properties in general, directly attack on the nervous system and damage it, primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae [106], act as mitochondrial poison [107] and work by interacting with cuticle membrane of the larvae ultimately disarranging the membrane which is the most probable reason for larval death [108]. This could be due to the presence of alkaloids, flavonoids, steroids, tannins, terpenes and terpenoids and it is said that several groups of the above mentioned phytochemicals from different plants have been reported for their insecticidal activities [18].

The promising effects of plant products on the pupal stage of *Aedes aegypti* are related to the morphological differences between the pupae and larvae of this insect, indicating that the mode of action of these products is by contact or choking and not swallowing, because the pupal stage does not involve

ingestion. The ethanolic extracts of *Haplophyllum tuberculatum* did not produce any ovicidal effect, but killed first instar larvae of *Culex quinquefasciatus* [109]. However, there are few studies of the effect of natural products on the pupal stage [81]. In the present study, the butanol extract was found to be effective in *Alternanthera sessilis*. Sun *et al*. [110] screened ethyl-acetate, n-butyl alcohol and water fractions of alcoholic extract of leaves and stems of *Vanilla fragrans* against *Culex pipiens* larvae and found both n-butyl alcohol and ethyl-acetate fractions to be active. The butanol extract of soapberry plant, *Phytolacca dodecandra* was very toxic to second and third instar larvae of *Aedes aegypti*, *Culex pipiens* and *Anopheles quadrimaculatus*, but eggs and pupae were unaffected [88].

The biological activity of crude extracts depends upon the mixture of active compounds. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. These products contain a complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans [111]. Tannins, alkaloids, steroids, glycosides, triterpenoids and saponins have been reported to be responsible for larval toxicity of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles* species mosquitoes [64,109,112]. The basic parameters influencing the quality of an extract are plant part, solvent used for extraction and extraction procedure. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction and diversity of different compounds extracted in the bioassay process [113]. The choice of solvent is influenced by what is intended with the extract. The choice will also depend on the targeted compounds to be extracted [111,114]. In the present study, in the case of larvicidal and pupicidal activity, the ethanolic extract was found to be the most active solvent since it could extract bioactive phytocompounds responsible for immature mosquitocidal activity. The activity of the ethanolic extracts can be attributed to the presence of higher amounts of polyphenols. It means that they are more efficient in cell walls and seeds degradation which have nonpolar character and cause polyphenols to be released from cells [115]. Ethanol has the property to extract tannins, polyphenols, polyacetylenes, flavonols, terpenoids, sterols and alkaloids [116]. The bioactive flavonoid compounds were detected with ethanol due to its higher polarity. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material [117].

Mosquito larvae of different species display different susceptibilities to the same phytochemicals. Selection of mosquito species for testing is also of fundamental importance since great variations exist in responses between the genera and species. Since aedines are usually less susceptible to insecticides and since *Aedes aegypti* is the most commonly colonized mosquito, it should be used for comparative screening. In general, *Aedes* larvae are more robust and less susceptible to insecticides and botanical extracts than *Culex* larvae. The generalization made with *Aedes* and *Culex* larvae does not always hold with *Anopheles* species. The susceptibility of *Anopheles* larvae can vary since they can be more or less susceptible than *Culex* and *Aedes* larvae to botanical derivatives and insecticides. However, *Aedes* species will not be the species of choice if a polluted environment involving decomposed leaf litter is being evaluated for activity, wherein *Culex quinquefasciatus* is preferable [18].

Plant bioactive components may serve as a suitable alternative to chemical insecticide as they are relatively safe and available everywhere in the world. The efficacy of botanicals however, generally depends on the plant part [118], extract concentration, age of plant or location found, solvent used and species of larvae tested [119-121]. The solvent used contribute to the variation since it has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents used [8]. Shaalan *et al*. [18] reported that screening involves mosquitocidal bioassay guided fractionation to identify highly active fractions and compounds isolated from the crude extract. The crude extract contains a complex mixture of biocidal active compounds. Hence, crude plant extracts have played an important role in this aspect. Several studies have documented the efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito larvae. Further, Tehri and Singh [122] stated that the successful results of preliminary studies on mosquitocidal potential of plant extracts encourage further effort to investigate the bioactive compounds in those extracts that might possess good immature mosquitocidal properties when isolated in pure form. In addition, novel drug delivery system of plant based active substances is the need of the hour.

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