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Bioefficacy of *Catharanthus roseus* (L.) G. Don (Apocyanaceae) and *Hyptis suaveolens* (L.) Poit (Lamiaceae) ethanolic aerial extracts on the larval instars of the dengue and chikungunya vector *Aedes aegypti* Linnaeus 1762 (Diptera: Culicidae)

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Abstract

Extensive use of synthetic and chemical insecticides has resulted in environmental hazards and also in development of physiological resistance among vector mosquito species. Plant products are considered to be a potential alternative approach as they are environmentally safe, target specific and biodegradable. In the present study, the bioefficacy of ethanolic extract of *Catharanthus roseus* and *Hyptis suaveolens* aerial parts were tested on the first, second, third and fourth instar larvae of *Aedes aegypti* at concentrations of 100, 200, 300, 400 and 500mg/L. Larval mortality was observed after 24 hours and the corresponding LC₅₀ values were 22.91, 28.30, 35.64 and 36.82mg/L and mortality recorded in terms of percentage were 96.86, 95.19, 98.53 and 98.53% at 500mg/L for *Catharanthus roseus*. For, *Hyptis suaveolens*, it was 68.36, 99.93, 101.24 and 114.93mg/L and 96.86, 93.52, 95.19% and 98.53 at 500mg/L respectively. Further investigations are needed to elucidate the larvicidal activity against a wide range of mosquito species and the active ingredient(s) responsible for larvicidal activity should be identified.

Keywords: *Catharanthus roseus*, *Hyptis suaveolens*, ethanolic aerial extract, larvicidal activity, *Aedes aegypti*

1. Introduction

Mosquitoes are popularly referred to as ‘flying syringes’, ‘tiny buzzing vampires’, ‘tiny assassins’^[1] and by World Health Organization^[2] as ‘public enemy number one’ are the worst enemy of mankind since dawn of time and act as a vector of several dreadful diseases. Till date, specific medications and vaccinations are not available commercially for treating dengue fever. The only approach followed to reduce the incidence of dengue is by the control of its vector, *Aedes aegypti*, which is also the primary carrier of chikungunya virus and yellow fever virus. In the past, the control measures for mosquito vectors were based on the frequent and indiscriminate use of synthetic chemical-based insecticides, viz., organochlorines, carbamates, organophosphates and pyrethroids^[3]. Nevertheless, the blind use of insecticides has resulted in the increased selection pressure on the mosquitoes leading to the development of insecticide resistance in them^[4, 5]. In addition, it has raised many other concerns including toxicity to human beings, harm to non-target population, long persistence in environment, and entry in the food chain^[6]. Keeping in view the increasing documentation of negative environmental and health impact of synthetic insecticides and increasingly rigorous environmental directives about use of pesticides, the researchers have transformed their interest towards the development and use of botanical pest management products for controlling mosquitoes and other insects^[7].

Botanicals are considered safe alternative to synthetic pesticides since they are biodegradable and safe for the environment causing low toxicity to humans and non-target organisms^[8]. Plant species have already been known to possess chemical factors and metabolites of significance in pest control programs whilst products of plant species have been reported to encompass diverse activities against mosquitoes^[9, 10].

A number of such plant products have been used for insect control since primordial time. Biologically active plant extracts have been well recognized for formulating an ecologically sound and environmentally accepted mosquito control program; several studies are being carried out to identify a variety of bioeffective substances found in different plant species [7]. A brief delve into the literature reveals many investigations have been made towards the biological screening of botanical extracts and the activity of many plant derived components against mosquitoes [9-17] 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 [18-28]. Thus, in continuation to the work by the above mentioned researchers, the present investigation was carried out to explore the larvicidal properties of crude ethanolic aerial extract of *Catharanthus roseus* and *Hyptis suaveolens* against the dengue vector, *Aedes aegypti* under laboratory conditions since reports were scanty with regard to the ethanolic aerial extract of the above mentioned plants.

2.0. Materials and methods

2.1. Plant collection and preparation of phytoextracts

Mature and healthy *Catharanthus roseus* (Figure 1) and *Hyptis suaveolens* (Figure 2) plants collected from Chennai, Tamil Nadu, India was taxonomical identified and confirmed at the Department of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai, Tamil Nadu, India. The aerial parts were then washed in dechlorinated water, shade dried and powdered with the aid of an electric blender. The powdered aerial parts (1Kg) of *Catharanthus roseus* was extracted with ethanol (3L) in a Soxhlet apparatus with minor modifications [29] and air dried to obtain the crude aerial extract which were stored in air tight amber coloured bottles at 4°C for bioassays. Likewise, the same methodology was adopted to obtain the crude ethanolic aerial extract of *Hyptis suaveolens*.



Fig 1: *Catharanthus roseus*



Fig 2: *Hyptis suaveolens*

2.2. Test mosquitoes

Cyclic generations of the *Aedes aegypti* mosquitoes, free of exposure to insecticides were maintained separately in mosquito cages (2'x2'x2') in an insectary with a mean room temperature of $27 \pm 2^\circ\text{C}$ 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 for adult emergence.

2.3. Larvicidal bioassay

Larvicidal bioassay was carried out as per the guidelines of World Health Organization [30] with minor modifications. Larvicidal activity at test concentrations of 100, 200, 300, 400 and 500mg/L of crude ethanolic aerial extract of *Catharanthus roseus* and *Hyptis suaveolens* were 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 first, second, third and fourth instar *Aedes aegypti* larvae from laboratory colonized mosquitoes of F₁ generation were introduced into glass beakers (250mL) each containing 200mL of distilled water and test concentration. Untreated control (distilled water only) and treated control (Tween 80 added to distilled water) were maintained separately and run simultaneously. Mortality was observed 24 hours after treatment. Moribund larvae were scored dead when they showed no signs of movement when probed by a needle at their respiratory siphon. 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 [31]. A total of five replicates per trial for each concentration were carried out. Statistical analysis of all mortality data of larvicidal activity were subjected to probit analysis [32]. The differences were considered as significant at $P \leq 0.05$ level.

Per cent larval mortality (1):

$$\frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Corrected percentage of control mortality (2):

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

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

3. Results

No larval mortality was observed in treated and untreated control. The crude ethanolic aerial extract of *Catharanthus roseus* exhibited larvicidal activity against the first, second, third and fourth larval instars of dengue vector mosquito after 24 hours of exposure and their the corresponding LC₅₀ values were 22.91, 28.30, 35.64 and 36.82mg/L and mortality recorded in terms of percentage were 96.86, 95.19, 98.53 and 98.53% respectively at 500mg/L. In the case of *Hyptis suaveolens*, the corresponding LC₅₀ values were 68.36, 99.93, 101.24 and 114.93mg/L and the percentage of larval mortality were 96.86, 93.52, 95.19 and 98.53% at 500mg/L respectively (Table 1; Figure 3).

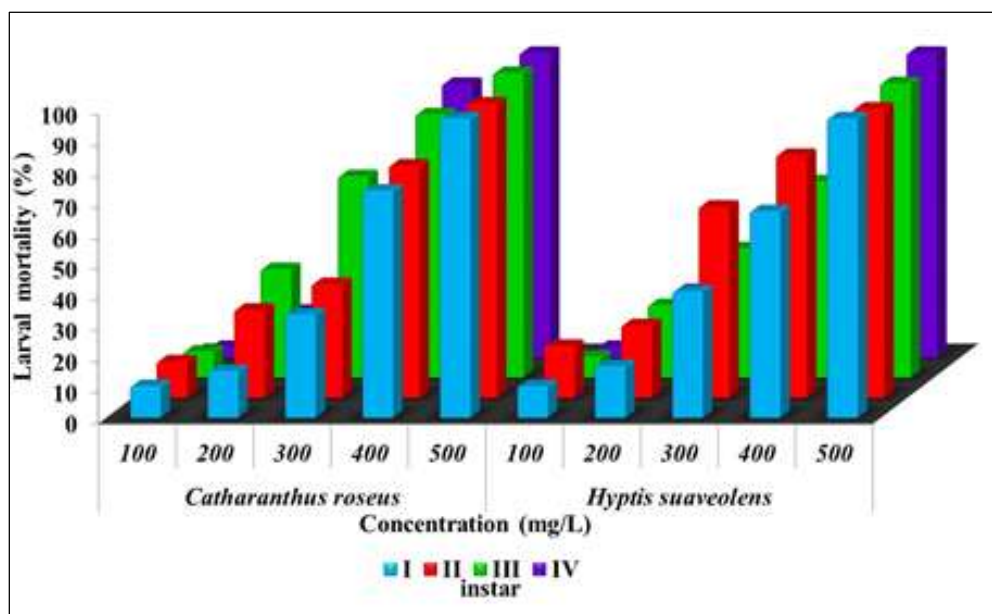


Fig 3: Per cent *Aedes aegypti* larval mortality on exposure to *Catharanthus roseus* and *Hyptis suaveolens* ethanolic aerial extracts

4. Discussion

Raveen *et al.* [27] has provided an exhaustive review on the larvicidal property of plants belonging to Apocynaceae family and *Catharanthus roseus* belongs to this plant family. The results of the present study can be corroborated with recent reports of *Catharanthus roseus* larvicidal efficacy. Remia and Logaswamy [33] studied the toxicity of *Catharanthus roseus* acetonetic leaf extract against the second and fourth instar larvae of *Aedes aegypti* with LC₅₀ values of 75.31 and 156.85mg/L respectively. The ethanolic leaf extract of *Catharanthus roseus* studied by Alam *et al.* [34] revealed LC₅₀ values of 150.0, 145.0 and 160.57mg/L against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* respectively. Subarani *et al.* [35] studied the larvicidal activity of *Catharanthus roseus* crude aqueous, ethyl acetate and methanolic leaf extract against *Anopheles stephensi* and *Culex quinquefasciatus*. Their corresponding LC₅₀ values were 68.62, 82.47, 78.80 and 85.21, 76.84, 94.20mg/mL respectively. In another study, Ekaputri *et al.* [36] indicated the ethanolic fruit extract of *Catharanthus roseus* for larvicidal efficacy with an LC₅₀ value of 2.99mg/mL against *Aedes aegypti*. Prasad *et al.* [37] pointed out that the methanolic leaf and flower extracts of *Catharanthus roseus* exhibited LC₅₀ values of 67.61 and 37.15mg/L against *Anopheles stephensi*. Kamatchi *et al.* [38] indicated the aqueous leaf extracts of

Catharanthus roseus to exhibit LC₅₀ values of 30.28, 38.01, 59.12, 71.81 and 26.64, 34.64, 53.10, 72.89mg/L against the first, second, third and fourth instars of *Culex quinquefasciatus* and *Aedes aegypti*. Sharma *et al.* [39] reported the hexane extracts of *Catharanthus roseus* leaves for larvicidal activity against *Aedes aegypti* and LC₅₀ value was 86.91mg/L. Pavunraj *et al.* [40] reported the hexane, ethyl acetate and methanolic leaf extracts of *Catharanthus roseus* with LC₅₀ values of 645.33, 1370.06 and 715.39mg/L against *Culex quinquefasciatus* respectively. Shoba *et al.* [41] pointed out that the ethanolic leaf extracts of *Catharanthus roseus* possesses an LC₅₀ value of 157.8mg/L against *Aedes aegypti* larvae.

Likewise, reports on the larvicidal efficacy of *Hyptis suaveolens* were compared with other studies. With respect to recent reports on the larvicidal efficacy of *Hyptis suaveolens*, Arivoli and Samuel [42] reported the hexane, diethyl ether, dichloromethane and ethyl acetate aerial extracts to exhibit larvicidal activity against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* with corresponding LC₅₀ values as 543.66, 1443.53, 1292.36, 853.04; 1523.19, 1490.78, 1396.41, 944.08; and 203.37, 888.00, 1321.05, 774.16mg/L respectively. Kovendan *et al.* [43] reported that the hexane, chloroform, ethyl acetate and methanolic leaf extracts of *Hyptis suaveolens* were active against the larvae of *Culex*

quinquefasciatus with respective LC₅₀ values of 213.09, 217.64, 167.59 and 86.93mg/L. Kovendan *et al.* [44] showed hexane, diethyl ether, ethyl acetate and acetic extracts of *Hyptis suaveolens* leaves to be active against the larvae of *Anopheles culicifacies* and their corresponding LC₅₀ values were 423.00, 347.50, 236.58 and 217.24mg/L. Ohimain *et al.* [45] revealed the methanol, chloroform and hexane leaf extracts of *Hyptis suaveolens* to be active against *Anopheles gambiae* larvae and LC₅₀ values were 73.25, 76.25 and 97.25mg/L respectively. Sakthivadivel *et al.* [46] indicated the crude petroleum ether, chloroform and acetone aerial extracts of *Hyptis suaveolens* for activity against *Culex quinquefasciatus* larvae and their respective LC₅₀ values were 493.44, 625.97 and 485.61mg/L. Mohankumar *et al.* [47] examined the methanolic leaf extracts of *Hyptis suaveolens* for larvicidal activity against *Aedes aegypti* and *Anopheles stephensi* with LC₅₀ values of 327.18 and 391.66mg/L. Ezihe *et al.* [48] revealed that the hexane leaf extracts of *Hyptis suaveolens* showed activity against larvae of *Culex quinquefasciatus* and its LC₅₀ value was 6.4%. Oumarou [49] reported the methanolic leaf extracts of *Hyptis suaveolens* to possess activity against *Anopheles gambiae* larvae with LC₅₀ value of 132.01mg/L.

Plants are rich sources of complex mixtures of bioactive compounds that can be used to develop environmentally safe vector and pest-managing agents. It could also be conceived from the review that some phytochemicals act as general toxicants both against adult as well as larval stages of mosquitoes. A number of researches in the field of vector control have revealed the efficacy of different phytochemicals obtained from various plants against different species of mosquitoes. Sukumar *et al.* [9] made an extensive review of botanical derivatives tested for mosquito control. Plant products can be obtained either from the whole plant or from a specific part (roots, bark, leaves, flowers, fruits and seeds) in their crude form by extraction with different types of non-polar, mid polar and polar solvents, *viz.*, hexane, petroleum ether, dichloromethane, diethyl ether, benzene, chloroform, acetone, ethyl acetate, methanol, ethanol, distilled water, etc.

Preliminary screening is a good means of evaluation of the potential mosquitocidal activity of plants used for this purpose. Komalamisra *et al.* [50] screened ninety-six ethanolic extracts from various parts of 84 Thai plant species for their larvicidal activity against *Aedes aegypti* mosquitoes of which extracts from *Rhinacanthus nasutus*, *Derris elliptica*, *Trigonostemon reidioides*, *Homalomena aromatica*, *Stemona tuberosa* and *Acorus calamus* possessed high larvicidal activity, with LC₅₀ values falling between 16.0 and 48.2mg/L. Das *et al.* [51] reported that the ethanolic extracts from *Aristolochia saccata* roots, *Annona squamosa* leaves and *Gymnopetelu cochinchinensis* fruits/pericarp against *Aedes albopictus* and *Culex quinquefasciatus* larvae ranged from 31.8 to 155.0ppm. The LC₅₀ values of 2.70, 11.33 and 12.54 mg/mL given by *Alstonia boonei* leaf extracts, respectively after 24 hours of exposure against *Anopheles arabiensis* indicated ethanol > aqueous > methanol extracts as the order of larvicidal activity [52]. Choochote *et al.* [53] reported that the ethanol extracted *Apium graveolens* did not cause rapid mortality, suggesting a delayed type of larval killing property. All larvae were active and exhibited a normal appearance with the siphon pointed up and head hung down. Nonetheless, the

symptoms caused by ethanol extracts were nerve poisons (excitation, convulsions, paralysis and death of the larvae) which was observed in the present study.

The solvent ethanol extracts alkaloids, anthraquinones, flavonoids, flavonols, phenols and polyphenols, polyacetylenes, saponins, steroids, sterols, tannins, terpenoids and triterpenoids [54]. Some of the ethanolic plant extracts reported for mosquito larvicidal activity are presented in Table 2. The higher 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 unpolar character and cause polyphenols to be released from cells [55]. The higher concentrations of more bioactive compounds were detected with ethanol due to its higher polarity [56]. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. The mortality of mosquito larvae might be caused by the secondary metabolites contained in the extracts of plant species. Alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids are among the metabolites with biological activities against insects [57] and for larval toxicity of mosquitoes in particular [58]. Plant secondary metabolites interfere with the proper functioning of mitochondria more specifically at the proton transferring sites. Secondary metabolites from different plants species cause physiological and cellular disturbances that include inhibition of acetylcholinesterase, disruption of sodium and potassium ion exchange, and interference of mitochondrial respiration [59]. Moreover, they affect midgut epithelium or gastric caecae and the malpighian tubules in mosquito larvae [60]. In addition, triterpenoids and saponins in chloroform; saponins in hexane; steroids, saponins, tannins and alkaloids in methanol extracts had revealed their toxicity against *Aedes aegypti* and *Culex quinquefasciatus* larvae [61]. Saponins and alkaloids had been reported by Mousumi *et al.* [62] to be responsible for toxicity on all instar larvae of *Culex quinquefasciatus*. 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 acetylcholinesterase (AChE) or sodium channels as inhibition of acetylcholinesterase activity is responsible for terminating the nerve impulse transmission through synaptic pathway [63,64]. 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 [65]. Shoba *et al.* [41] reported presence of alkaloids, terpenoids, flavonoids, tannins and saponins when the ethanolic leaf extract of *Catharanthus roseus* was subjected to phytochemical screening. In the present study, it is anticipated that ursolic acid, a triterpenoid compound from *Catharanthus roseus* would have been responsible for the larval mortality since da Silva *et al.* [66] have reported for the first time the larvicidal properties of the triterpenoid, ursolic acid and their derivatives against *Aedes aegypti*. Further, da Silva *et al.* [66] reported that the structural characteristics that contribute to the understanding of the larvicidal activity of triterpene compounds were identified wherein the presence of a hydroxyl group is essential for larvicidal potential. Therefore, more investigations on

evaluation, identification and isolation of the bioactive phytocomponent(s) are necessary. In conclusion, the results of the present study marked a larvicidal effect in the ethanolic aerial extract of *Catharanthus roseus* when compared with *Hyptis suaveolens*. Further studies of the active principles involved and their mode of action, formulated preparations for

enhancing potency and stability, toxicity and effects on non-target organisms and the environment, and field trials are needed to recommend phytopesticides as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

Table 1: Larvicidal activity of ethanolic aerial extracts against instars of *Aedes aegypti*

Instars	LC ₅₀ (mg/L)	95% confidence limit		RA	Df	χ ²
		LL	UL			
<i>Catharanthus roseus</i>						
I	22.91	14.04	39.95	Y = 1.5878 + 2.5612 log X	4	9.2*
II	28.30	23.81	33.71	Y = 1.6677 + 2.3220 log X	4	6.3*
III	35.64	30.45	41.41	Y = 0.6726 + 2.7738 log X	4	1.0*
IV	36.82	32.30	42.02	Y = -0.1993 + 3.3485 log X	4	3.5*
<i>Hyptis suaveolens</i>						
I	68.36	58.16	80.85	Y = 0.4415 + 2.4782 log X	4	6.6*
II	99.93	83.21	119.17	Y = -1.156 + 3.0163 log X	4	3.3*
III	101.24	85.74	120.28	Y = 0.7051 + 2.1426 log X	4	4.3*
IV	114.93	98.94	133.92	Y = 0.0777 + 2.4780 log X	4	5.8*

LC₅₀: lethal concentration that kills 50% of the exposed larvae; LC₉₀: lethal concentration that kills 90% of the exposed larvae; LL-lower limit; UL-upper limit; RA-regression analysis; Df: degree of freedom; *P<0.05 level of statistical significance of (χ²) chi-square values

Table 2: List of ethanolic plant extracts reported for mosquito larvicidal activity

Plant species	Family	Part	LC ₅₀	Mosquito species	Work cited
<i>Acacia concinna</i>	Fabaceae	Fruit	162.59mg/L	AA	Komalamisra <i>et al.</i> [50]
<i>Acorus calamus</i>	Araceae	Root	48.24mg/L		
<i>Allium sativum</i>	Amariyllidaceae	Bulb	0.0727mg/mL	CQ	Suryadevara and Khanam [67]
<i>Alstonia boonei</i>	Apocynaceae	Leaf	2.70mg/mL	AAR	Omoya <i>et al.</i> [52]
<i>Alternanthera philoxeroides</i>	Amaranthaceae	Whole	155.37mg/L	AA	Devi and Bora [68]
<i>Ammi visnaga</i>	Apiaceae		0.42mg/mL	CP	Yassine <i>et al.</i> [69]
<i>Amphineuron opulentum</i>	Thelypteridaceae	Fronnd	104.00mg/L	AA	Devi and Bora [52]
<i>Anacardium occidentale</i>	Anacardiaceae	Shell	2.35mg/L		Torres <i>et al.</i> [70]
<i>Andrographis echioides</i>	Acanthaceae		Leaf		3.29mg/L
<i>Annona crassiflora</i>	Annonaceae	108.3mg/L			Rajkumar <i>et al.</i> [72]
<i>Annona crassiflora</i>		0.71µg/mL	Omena <i>et al.</i> [73]		
<i>Annona glabra</i>		Root			0.71µg/mL
<i>Annona muricata</i>		Seed			0.06µg/mL
<i>Annona reticulata</i>		Root			42.3µg/mL
<i>Annona squamosa</i>		Leaf			330.51mg/L
		Seed			20.26µg/mL
		Leaf		132.63mg/L	
		Root		31.9µg/mL	
		Leaf	101.96mg/L		
		20.70	AAL	Das <i>et al.</i> [51]	
<i>Apium graveolens</i>	Umbelliferae	Seed	6.00mg/mL	CQ	Shad and Andrew [76]
<i>Aristolochia saccata</i>	Aristolochiaceae	Root	81.0mg/L	AA	Choochate <i>et al.</i> [53]
<i>Azadirachta indica</i>	Meliaceae	Leaf	17.30	AAL	Das <i>et al.</i> [51]
			390.0mg/L	CF	Azmi <i>et al.</i> [77]
			8.32mg/mL	AA	Mgbemena [78]
		1.805mg/mL	CQ	Khan <i>et al.</i> [79]	
		Fruit endocarp	0.034g%	AA	Wandscheer <i>et al.</i> [80]
		Seed	15.495µg/mL	CQ	Mandal [81]
<i>Cadaba indica</i>	Capparaceae	Leaf	144.50mg/L	AA	Kovendan <i>et al.</i> [82]
<i>Cadaba trifoliata</i>			123.4mg/L		Rajkumar <i>et al.</i> [72]
<i>Calotropis gigantea</i>	Asclepiadaceae	Fruit	183.07mg/L		Komalamisra <i>et al.</i> [50]
<i>Capsicum frutescens</i>	Solanaceae		231.59mg/L		Alvarez <i>et al.</i> [83]
			300.20mg/L	AAL	
			100.0%	Alvarez <i>et al.</i> [84]	
<i>Cardiospermum halicacabum</i>	Steminaceae	Leaf	543.19mg/L	AA	Komalamisra <i>et al.</i> [50]
<i>Carica papaya</i>	Caricaceae	Root	36%		Malathi and Vasugi [85]
<i>Caryota bacsonensis</i>	Palaceae	Fruit	155.65mg/L		Komalamisra <i>et al.</i> [50]

<i>Cassia mimosoides</i>	Caesalpinaceae	Leaf and Pod	4.85mg/mL	AG	Alayo <i>et al.</i> [86]	
<i>Cassia obtusifolia</i>	Leguminosae	Leaf	52.2mg/L	AS	Rajkumar and Jebanesan [87]	
<i>Cerbera odollum</i>	Apocynaceae		96.16mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Cerbera peruviana</i>		Fruit	150.33mg/L			
<i>Centella asiatica</i>	Umbelliferae	Leaf	6.84mg/L	CQ	Rajkumar and Jebanesan [88]	
<i>Chromolaena odoratum</i>	Asteraceae		433.88mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Chrysanthemum cinerariaefolium</i>			187.78mg/L	AG	Araka <i>et al.</i> [89]	
<i>Cinnamomum rhyncophyllum</i>	Lauraceae	Fruit	188.64mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Citrullus colocynthis</i>	Cucurbitaceae	Fruit pulp	25.12mg/L	AAR	Hamid <i>et al.</i> [90]	
<i>Citrus citratus</i>	Rutaceae	Leaf	34.67mg/mL	AA	Mgbemena [78]	
<i>Citrus hystrix</i>			1.183mg/mL		Mya <i>et al.</i> [91]	
<i>Citrus reticulata</i>		Seed	2267.71mg/L	CQ	Sumroiphon <i>et al.</i> [92]	
<i>Clausena anisata</i>			112.7mg/L	AAR	Mavundza <i>et al.</i> [93]	
<i>Combretum quadrangulare</i>			722.29mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Cosmos bipinnatus</i>	Asteraceae	Leaf	1.18mg/mL	CQ	Modise and Ashafa [94]	
<i>Crinum asiaticum</i>	Amaryllidaceae	Fruit	177.76mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Croton tiglium</i>	Euphorbiaceae	Root	60.87mg/L		Komalamisra <i>et al.</i> [50]	
<i>Cryptomeria japonica</i>	Cupressaceae	Leaf	60.1µg/mL		Gu <i>et al.</i> [95]	
<i>Curcuma longa</i>	Zingiberaceae		106.38mg/L		Komalamisra <i>et al.</i> [50]	
<i>Curcuma mangga</i>		Fruit	106.38mg/L		Sukari <i>et al.</i> [96]	
<i>Curcuma odollum</i>		Rhizome	133.7mg/L		Komalamisra <i>et al.</i> [50]	
<i>Curcuma zedoaria</i>		Fruit	102.23mg/L		Komalamisra <i>et al.</i> [50]	
<i>Datura stramonium</i>		Solanaceae	Seed		93.38mg/L	Swathi <i>et al.</i> [97]
<i>Derris species</i>	Leguminosae	Leaf	86.25mg/L		Omena <i>et al.</i> [73]	
<i>Derris elliptica</i>	Fabaceae		Root		8.54µg/mL	Komalamisra <i>et al.</i> [50]
<i>Derris scandens</i>			Fruit	20.49mg/L	Komalamisra <i>et al.</i> [50]	
<i>Eclipta paniculata</i>	Asteraceae	Aerial	122.90mg/L	Macedo <i>et al.</i> [98]		
<i>Emblica officinalis</i>	Phyllanthaceae	Fruit	3.3mg/L	AF	Uthayarasa <i>et al.</i> [99]	
<i>Erythrina mulungu</i>	Leguminosae	Stem bark	239.35mg/L	AA	Omena <i>et al.</i> [73]	
<i>Eucalyptus camaldulensis</i>	Myrtaceae	Leaf	67.9µg/mL	AG	Araka <i>et al.</i> [89]	
<i>Eucalyptus citriodora</i>			210.15mg/L	AA	Uthayarasa <i>et al.</i> [99]	
<i>Eucalyptus globulus</i>			188.99mg/L		Alvarez <i>et al.</i> [83]	
<i>Euphorbia antiquorum</i>			62.22%		Komalamisra <i>et al.</i> [50]	
<i>Euphorbia pulcherrima</i>	599.74mg/L	Euphorbiaceae	548.94mg/L		310.56mg/L	
<i>Euphorbia tirucalli</i>	121.24mg/L			AS		Elangovan <i>et al.</i> [100]
<i>Exacum pedunculatum</i>	0.10mg/mL			CQ		Modise and Ashafa [94]
<i>Foeniculum vulgare</i>	Apiaceae	Fruit	5.52mg/L	AA	Torres <i>et al.</i> [101]	
<i>Garcinia mangostana</i>	Clusiaceae	Dried exudate	3.52mg/mL	CQ	Suryadevara and Khanam [67]	
<i>Gardenia gummifera</i>	Rubiaceae	Leaf	241.64mg/L	AA	Patil <i>et al.</i> [102]	
<i>Gossypium hirsutum</i>	Malvaceae	Root	38.10mg/L		Komalamisra <i>et al.</i> [50]	
<i>Homalomena aromatica</i>	Araceae	Leaf	542.88mg/L		Devi and Bora [52]	
<i>Homalomena rubescens</i>			241.73mg/L		Alvarez <i>et al.</i> [89]	
<i>Hedyclium coronarium</i>	Zingiberaceae	Whole	585.10mg/L		Aina <i>et al.</i> [103]	
<i>Ipomoea aquatica</i>	Convolvulaceae	Leaf	88.89%		Khan <i>et al.</i> [79]	
<i>Jatropha curcas</i>	Euphorbiaceae	Seed	3.25mg ml ⁻¹		Wandscheer <i>et al.</i> [80]	
<i>Melia azedarach</i>	Meliaceae	Leaf	1.949mg/mL	AA	Komalamisra <i>et al.</i> [50]	
<i>Mentha longifolia</i>		Lamiaceae	Fruit endocarp	0.017g%	Cetin <i>et al.</i> [104]	
<i>Mentha pulegium</i>			Seed	76.69mg/L		
<i>Morinda citrifolia</i>	Rubiaceae	Aerial	26.8mg/L	CP	Kovendan <i>et al.</i> [105]	
<i>Murraya koenigii</i>	Rutaceae	Leaf	81.0mg/L	AS	Alvarez <i>et al.</i> [83]	
<i>Nerium oleander</i>	Apocynaceae		237.43mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Nicotiana tabacum</i>	Solanaceae		71.11%	AG	Araka <i>et al.</i> [89]	
<i>Ocimum gratissimum</i>	Lamiaceae		197.97mg/L	AA	Mgbemena [78]	
<i>Pelagonium graveoleus</i>	Geraniaceae		19.50mg/mL	AG	Ofoegbu <i>et al.</i> [106]	
<i>Persea americana</i>	Lauraceae		Bark	60.9mg/mL	AA	Jennifer <i>et al.</i> [107]
<i>Persea membranacea</i>			Seed	1.36mg/L	AAL	Carvalho [108]
<i>Phyllanthus niruri</i>		Root	4.2mg/L	AV	Nzelibe and Albaba [109]	
<i>Physalis angulata</i>	Solanaceae	Fruit	53.72mg/L	AA	Omena <i>et al.</i> [73]	
<i>Pinus merkusii</i>	Pinaceae	Bark	11.92mg/L	AG	Prabakaran and Rajalakshmi [110]	
			2.50mg ml ⁻¹	AG	Aina <i>et al.</i> [103]	
			58.4mg/L	AA	Setiawan <i>et al.</i> [111]	

<i>Piper betle</i>	Piperaceae	Fruit	177.62mg/L	AG	Komalamisra <i>et al.</i> [50]	
<i>Piper guineense</i>		Seed	0.028mg ml ⁻¹		Aina <i>et al.</i> [103]	
<i>Piper longum</i>		Fruit endocarp	2.23mg/L		AA	Chaithong <i>et al.</i> [112]
<i>Piper ribesoides</i>			8.13mg/L			
<i>Piper sarmentosum</i>			4.06mg/L			
<i>Plumbago indica</i>	Plumbaginaceae	Leaf	202.21mg/L	Komalamisra <i>et al.</i> [50]		
<i>Pterodon polygalaeiflorus</i>	Leguminosaeae	Seed	35.7µg/mL	Omena <i>et al.</i> [73]		
<i>Pueraria candollei</i>	Fabaceae	Leaf	272.38mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Rhinacanthus nasutus</i>	Acanthaceae	Root	16.04mg/L			
		Stem	190.29mg/L			
<i>Rhizophora mucronata</i>	Rhizophoraceae	Bark	157.4mg/L	Kabaru and Gichia [113]		
<i>Ricinus communis</i>	Euphorbiaceae	Leaf	523.13mg/L	AAR	Komalamisra <i>et al.</i> [50]	
			1108.0mg/L			Basheer [114]
<i>Salvia sclarea</i>	Lamiaceae	Aerial	62.7mg/L	CP	Cetin <i>et al.</i> [104]	
<i>Sphaerostephanos unicus</i>	Thelypteridaceae	Fronde	292.15mg/L	AA	Devi and Bora [52]	
<i>Stemona tuberosa</i>	Stemineaceae	Root	43.48mg/L		Komalamisra <i>et al.</i> [50]	
<i>Sapindus rarak</i>	Sapindaceae	Seed	88.08mg/L			
<i>Sphaeranthus africanus</i>	Asteraceae	Leaf	260.66mg/L			
			573.80mg/L			
<i>Strophanthus caudatus</i>	Apocynaceae					
<i>Tagetes erecta</i>	Asteraceae	Flower	918.63µg/mL		Raj and Shettu [115]	
<i>Tagetes minuta</i>		Aerial	1.0mg/L	AF	Macedo <i>et al.</i> [98]	
		Leaf	1.17mg/mL	CQ	Modise and Ashafa [94]	
<i>Teucrium divaricatum</i>	Lamiaceae	Aerial	18.6mg/L	CP	Cetin <i>et al.</i> [104]	
<i>Thithonia diversifolia</i>	Asteraceae	Leaf	326.87mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Tribulus terrestris</i>	Zygophyllaceae		376.4mg/L		El-Sheikh <i>et al.</i> [116]	
<i>Trigonostemon reidioides</i>	Euphorbiaceae		Root		40.89mg/L	Komalamisra <i>et al.</i> [50]
<i>Turbinaria conoides</i>	Sargassaceae	Whole	64.27mg/L	AS	Valentina <i>et al.</i> [117]	
			88.18mg/L			
			74.45mg/L			CQ
<i>Vetiveria zizanioides</i>	Poaceae	Leaf	380.73mg/L	AA	Komalamisra <i>et al.</i> [50]	
<i>Vitex negundo</i>	Lamiaceae		848.24mg/L	Vijayakumar <i>et al.</i> [118]		
<i>Xylopiya aethiopia</i>	Annonaceae	Fruit	3.57mg ml ⁻¹	AG	Aina <i>et al.</i> [103]	
<i>Zanthoxylum nitidum</i>	Rutaceae	Stem bark	6.10mg/L	AA	Devi and Bora [52]	
<i>Zingiber amaricans</i>	Zingiberaceae	Fruit	188.08mg/L		Komalamisra <i>et al.</i> [50]	
<i>Zingiber officinale</i>		Leaf	270.60mg/L			
<i>Zingiber purpureum</i>		Root	64.02mg/L			
<i>Ziziphus jujuba</i>	Rhamnaceae	Leaf	79.98mg/L		Devi and Bora [52]	

AA: *Aedes aegypti*; AAL: *Aedes albopictus*; AV: *Aedes vittatus*; AF: *Anopheles fluviatilis*; AG: *Anopheles gambiae*; AAR: *Anopheles arabiensis*; AS: *Anopheles stephensi*; CF: *Culex fatigans*; CP: *Culex pipiens*; CQ: *Culex quinquefasciatus*

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