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LARVICIDAL EFFICACY OF MEDICINAL PLANT EXTRACTS FOR THE CONTROL OF MOSQUITOE VECTORS

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ABSTRACT

The larvicidal activities to determine the efficacies of acetone, ethyl acetate, chloroform and butanol dried leaf extracts of *Annona squamosa*, *Cynodon dactylon*, *Melia azedarach* and root extract of *Hemidesmus indicus* tested against 3rd instar *Culex quinquefasciatus* and *Aedes aegypti*. The highest larvae mortality was found in the acetone followed by ethyl acetate, chloroform and butanol extract of *A. squamosa* with LC_{50} values of 594.60, 490.35, 143.90, 117.98 and 280.62, 343.89, 156.92, 471.94 ppm; *C. dactylon* with LC_{50} values of 585.86, 143.32, 74.33, 148.65 and 408.48, 198.43, 169.28, 385.55 ppm; *M. azedarach* with LC_{50} values of 264.87, 65.27, 88.39, 514.65 and 192.78, 148.65, 172.24, 235.97 ppm *H. indicus* with LC_{50} values of 210.22, 174.82, 117.98, 445.45 and 363.91, 255.47, 186.61, 338.56 ppm against *C. quinquefasciatus* and *A. aegypti*. Our result suggested that the ethyl acetate of *M. azedarach* leaf extract was an excellent larvicidal potential in controlling filariasis and dengue vectors.

KEYWORDS: **Medicinal plants, *Culex quinquefasciatus*, *Aedes aegypti*, Lethal concentration**

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1. INTRODUCTION

Mosquitoes are one of the most important group of insects transmit dreadful diseases like dengue fever, yellow fever, chikungunya, malaria, encephalitis and filariasis. It has become a problem in many parts of the world, especially tropical and subtropical never ending countries [1, 2]. *Aedes aegypti* mosquito is a vector of many diseases in humans like yellow fever and dengue [3]. In field of vector control insecticide application proved to be effective against *A*. *aegypti*, is still facing a threat due to the development of resistance against chemical insecticides, leads to rebounding vectorial capacity [4]. Plants possess rich source of secondary metabolites, inclusive of mosquitocidal propertiesapart from to their biodegradable capacity they are considered good candidates for controlling mosquitoes [5]. Lymphatic filariasis (LF) is a major public health problem in India and worldwide, it is estimated that 1.39 billion people from 73 countries are living at the risk of infection. *C. quinquefasciatus* is the main vector of bancroftian filariasis. In India, it was reported that nearly 31 million microfilareamics and 23 million cases of symptomatic filariasis cases [6]. *C*. *quinquefasciatus* is an important vector of west nile virus and filarial nematode *Wuchereria bancrofti* [7]. *A*. *aegypti* larval toxicity bioassay was performed on compounds representing many classes of natural compounds, including flavonoids, sesquiterpenoids and triterpenoids [8]. Rajkumar and Jebanesan [9] reported that the bioactivity of four flavonoid compounds extracted from *Poncirus trifoliata* were studied for larvicidal, ovicidal and oviposition activities against the *A*. *aegypt*i. The cercaricidal activity was attributed to the presence of terpenoids and flavonoids in the extract of *Origanum compactum* [10]. Four identified isoflavonoids, isolated from wild relatives of chickpea, *Cicer arietinum* were shown to larval feeding by *Heliocoverpa armigera* [11]. Alpha-terthienyl a naturally occurring secondary plant metabolite is found in abundance in the roots of Tagetes species possess all the desirable properties of a good insecticide/pesticide [12]. The acetone extracts of *Ageratum conyzoides*, *Cleome icosandra*, *Tagete serectes* and *Tridax procumbens* showed growth inhibitory and juvenile hormone mimicking activity against *C*. *quinquefasciatus* [13]. The major constituents identified in the members of the Annonaceae are typically acetogenins that exhibited active in vitro anti-malarial activities [14, 15]. Das *et al* [16] reported that the ethanol leaf extract of *A*. *squamosa* found to have the most promising larvicidal activity against *C*. *quinquefasciatus* larvae. The hexane, chloroform, ethyl acetate, acetone and methanol extracts of the bark of *A*. *squamosa* have been reported as very active agents against the fourth-instar larvae of the malaria vector *A*. *stephensi* and the lymphatic filariasis vector *C*. *quinquefasciatus* [17].The larvicidal and mosquitocidal activities of ethanolic water mixture extract of *A*. *squamosa* and *Centella asiatica* leaves methanolic extract and the seeds of petroleum ether extract showed larvicidal activity tested against *A*. *stephensi*, *C*. *quinquefasciatus* and *A*. *aegypti* [18, 19]. The methanolic extracts of the leaves and seeds from the *M*. *azedarach* were tested against mature and immature mosquito vector *A*. *stephensi*. Ethanol extracts of different fraction of leaves and unripe fruit extracts of *M*. *azedarach* proved as an effective repellent against the eggs and nymphs of *Triatoma infestans*, the vector of Chagas disease [20]. The hexane, chloroform, ethyl acetate, acetone and methanol leaf, *C*. *dactylon* were tested against fourth instar larvae of *Anopheles vagus*, *Armigeres subalbatus* and *Culex vishnui* [21]. *Hemidesmus indicus* commonly known as Anantmul is a well-known drug in Ayurveda system of medicine [22]. *H. indicus* has long been used as a folk medicine and found to be an ingredient in ayurvedic and unani preparations which are usually prescribed against inflammation, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, urinary disorders, loss of appetite, burning sensation and rheumatism and especially for epileptic fits in children [23].The ethanol, methanol, chloroform and aqueous *H*. *indicus* extract were tested against *C*. *quinquefasciatus* [24].

2. MATERIALS AND METHODS

2.1. Collection of plant materials

The leaf of *A*. *squamosa* L. (Annonaceae) bermudagrass *C*. *dactylon* L. Pers. (Poaceae) leaf of *M*. *azedarach* L.(Meliaceae) and root of *H*. *indicus* L. R.Br. (Apocynaceae) were collected in and around Karadimalai, Vellore district, Tamil Nadu and the taxonomic identification was done by Prof. P. Muthumary, Centre for advanced Botany, University of Madras, Guindy campus, Chennai, India.

2.2. Extraction

The leaves were washed with double distilled water and shade-dried at room temperature (28±2 °C). The shade dried materials were powdered separately using a commercial electrical blender. The finely ground plant material (1,000 g/solvent) was filled in Soxhlet apparatus and was extracted with acetone, ethyl acetate, chloroform and butanol individually. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of these plants varies with the solvents used. One gram of the crude extract was first dissolved in 100 ml of respective solvent (stock solution). From this stock solution\ different concentrations were prepared used for larvicidal bioassay.

2.3. Insect rearing and bioassay

C. *quinquefasciatus* and *A*. *aegypti* larvae were collected from the stagnant water bodies and ditches located in Tambaram. The larvae were kept in plastic and enamel trays filled with tap water, for the maintained and reared in the laboratory as per the methods of Kamaraj et al [14]. For the laboratory trials the larvae of *A*. *aegypti* and *C*. *quinquefasciatus* were collected from the insect rearing cage and identified in Zonal Entomological Research Centre, Vellore, (Fig. 1A and B).

2.4. Preparation of the extract

From the stock solution 1,000 ppm was prepared with dechlorinated tap water. The larvicidal activity was assessed according to given in the procedure WHO (1996) with slight modifications and as per the method of Rahuman *et al* [25]. For the bioassay tests, larvae were sorted out in five batches of 20 in 249 ml of water and 1.0 ml of the desired plant extract concentration. From the stock solution, different concentrations 62.5,125, 250, 500 and 1,000 ppm were prepared for *A*. *squmosa*, *C*. *dactylon*, *M*. *azedarach* and *H*. *indicus* extracts. The control prepared with respective solvent simultaneously. The numbers of dead larvae were counted after 24 hrs of exposure and the percentage mortality was reported from the average of three replicates. The experimental medium, if 100% mortality of larvae occurred that particular dose was selected for further dose response bioassay.

2.5. Data analysis

The larval mortality data were subjected to probit analysis for LC_{50} calculation other statistic analysis at 95% fiducial limits of upper confidence limit and lower confidence limit and chi-square values were calculated by using the software developed by Reddy et al [26]. Results with $p<0.05$ were considered to be statistically significant.

3. RESULTS AND DISCUSSION

The preliminary screening is a good mode of evaluation of the potential larvicidal activity of plants popularly used for bioassay. Larvicidal activity of different solvent crude extracts of four plants are given in the Table 1. The larval percentage mortality showed in acetone, ethyl acetate, chloroform and butanol extracts of *A*. *squamosa, C*. *dactylon*, *M*. *azedarach* and *H*. *indicus* against *C*. *quinquefasciatus* at 62.5 to 1,000 ppm (Fig. 2). It was evident that all the extracts showed moderate to low larvicidal effects, however, the highest larval mortality was found in acetone, ethyl acetate, chloroform and butanol extracts of *A*. *squamosa* (LC_{50}= 594.60, 367.60, 115.02 and 81.01ppm), *C*. *dactylon* extracts (LC_{50}= 452.60, 106.92, 56.65 and 114.17 ppm), *M*. *azedarach* extracts of (LC_{50}= 215.85, 43.45, 54.28 and 227.02 ppm) and *H*. *indicus* extracts of (LC_{50}= 160.91, 119.61, 81.01 and 311.07 ppm) against the larvae of *C*. *quinquefasciatus*, Table 2. The

ethyl acetate leaf extracts of *Citrullus colocynthis* and *Cucurbita maxima* showed that the LC_{50} values were 47.58, and 75.91ppm, against larvae of *C*. *quinquefasciatus*. The ethyl acetate leaves extract of *O*. *sanctum* showed significant mortality against *A*. *aegypti* and *C*. *quinquefasciatus* with LC_{50} values of 425.94 and 592.60 ppm [27]. Elango *et al* [28] reported that ethyl acetate extract of the leaves of *Aegle marmelos* exhibited high larvicidal properties against *A. subpictus* and *Culex tritaeniorhynchus* having LC_{50} values of 167.00 and 99.03 ppm. Sharma et al. [29] had been reported that the acetone extract of *Nerium indicum* and *Thuja orientelis* studied with LC_{50} values of 200.87, 127.53, 209.00 and 155.97 ppm against third instar larvae of *A. stephensi* and *C. quinquefasciatus*. The ethyl acetate extracts of *Hyptis suaveolens* exhibited insecticidal and pesticidal properties [30]. Percent mortality shown in acetone, ethyl acetate, chloroform and butanol extracts of *A*. *squamosa*, *C*. *dactylon*, *M*. *azedarach* and *H*. *indicus* against *A*. *aegypti* at 62.5 to 1,000 ppm (Fig 4). Lethal concentration 50% were acetone, ethyl acetate, chloroform and butanol extracts of *A*. *squamosa* (LC_{50}= 280.62, 343.39, 156.92 and 471.94 ppm), *C*. *dactylon* extracts of (LC_{50}= 408.48, 198.43, 169.28 and 385.55 ppm), *M*. *azedarach* extracts of (LC_{50}= 192.78, 148.65, 172.24 and 235.97 ppm) and *H*. *indicus* extracts of (LC_{50}= 273.56, 255.47, 186.61 and 338.56 ppm) against the larvae of *C*. *quinquefasciatus* (Table 2). Kovendan *et al* [31] have reported the leaf extract of *A*. *alnifolia* with different solvents chloroform, ethyl acetate and acetone were tested for larvicidal activity of against *A*. *aegypti* LC_{50}=182.58, 160.35 and 146.07 ppm and LC_{90} = 460.83, 440.78 and 415.38 ppm and *C*. *quinquefasciatus* LC_{50}= 172.48, 151.06 and 140.69 ppm and LC_{90}= 430.66, 418.78 and 408.83 ppm. The larvicidal potential of acetone solvent crude leaf extracts *Elaeagnus indica*, *Maesa indica* tested against the fourth-instar larvae of *A*. *aegypti* (LC_{50} = 90.89 and 173.21ppm) [32]. The carbon tetrachloride extract of *S. xanthocarpum* was most effective petroleum ether extract was toxic with LC_{50} values of 62.62 ppm after 24 hrs and 59.45 ppm after 48 h of exposure period against *C*. *quinquefasciatus* [33]. The essential oil of *Ipomoea cairica* possessed remarkable larvicidal properties as it could produce 100% mortality in the larvae of *C. tritaeniorhynchus*, *A. aegypti* and *C. quinquefasciatus* mosquitoes at concentrations ranging from 100 to 170 ppm [34]. The larvicidal activity of petroleum ether, ethanolic and aqueous extracts of dried leaves and fixed oil from the seeds of *Caesalpinia bonduc* showed 100% mortality in 1% concentration of petroleum ether and ethanolic extract of leaves whereas it was 55% in 2.5% concentration of aqueous extract and 92.6% in 2.5% concentration of fixed oil against the fourth instar larvae of *C*. *quinquefasciatus* [35]. Rahuman *et al*. [25] reported the compounds 4-gingerol (1), (6)-dehydrogingerdione (2) and (6)-dihydrogingerdione (3) isolated from petroleum ether extract of *Zingiber officinale* exhibited larvicidal activities against fourth instar larvae of *A*. *aegypti* (LC_{50}=4.25, 9.80, and18.20 ppm) and *C*. *quinquefasciatus* (LC_{50}=5.52, 7.66 and 27.24 ppm), respectively.

Figure 1
(A) Culex quinquefasciatus and (B) Aedes aegypti

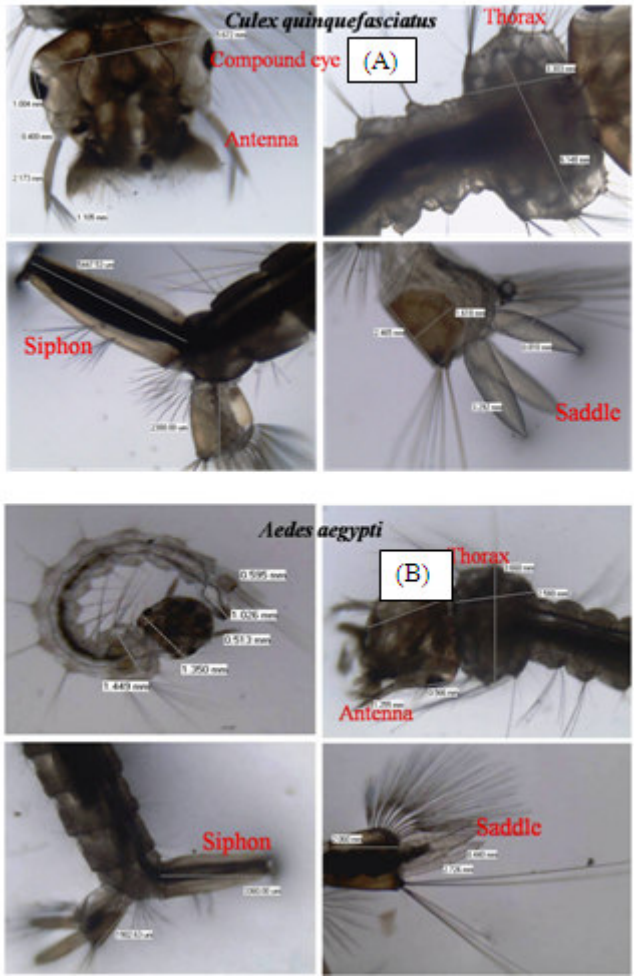

Figure 2

Larvicidal activity of crude extracts against C. quinquefasciatus (A) Annona squamosa, (B) Cynodon dactylon (C) Melia azedarach (D) Hemidesmus indicus

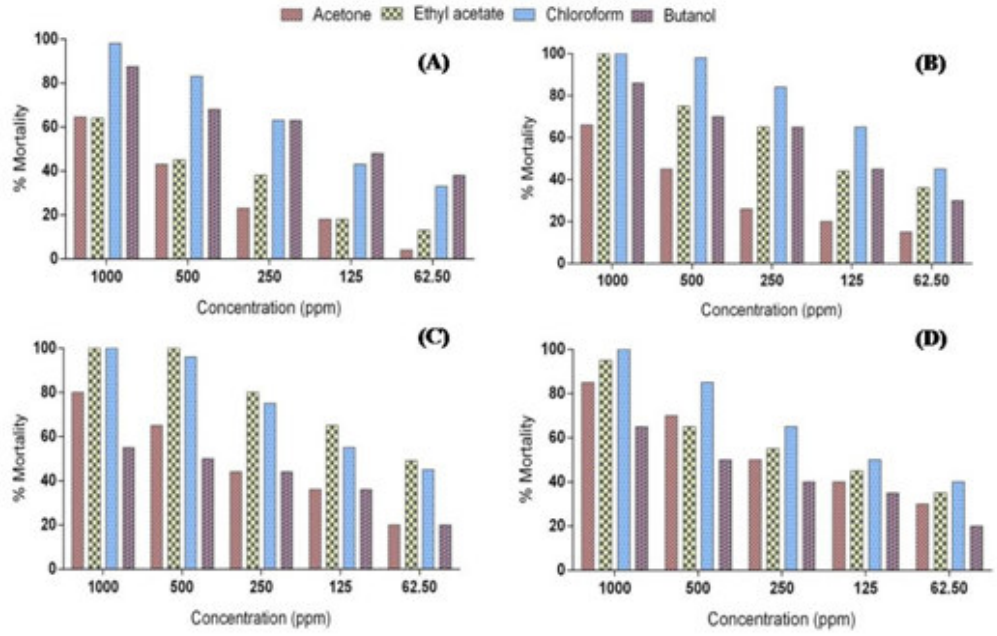

Figure 3

Larvicidal activity of crude extracts against A. Aegyptii ppm (A) Annona squamosa, (B) Cynodon dactylon (C) Melia azedarach (D) Hemidesmus indicus

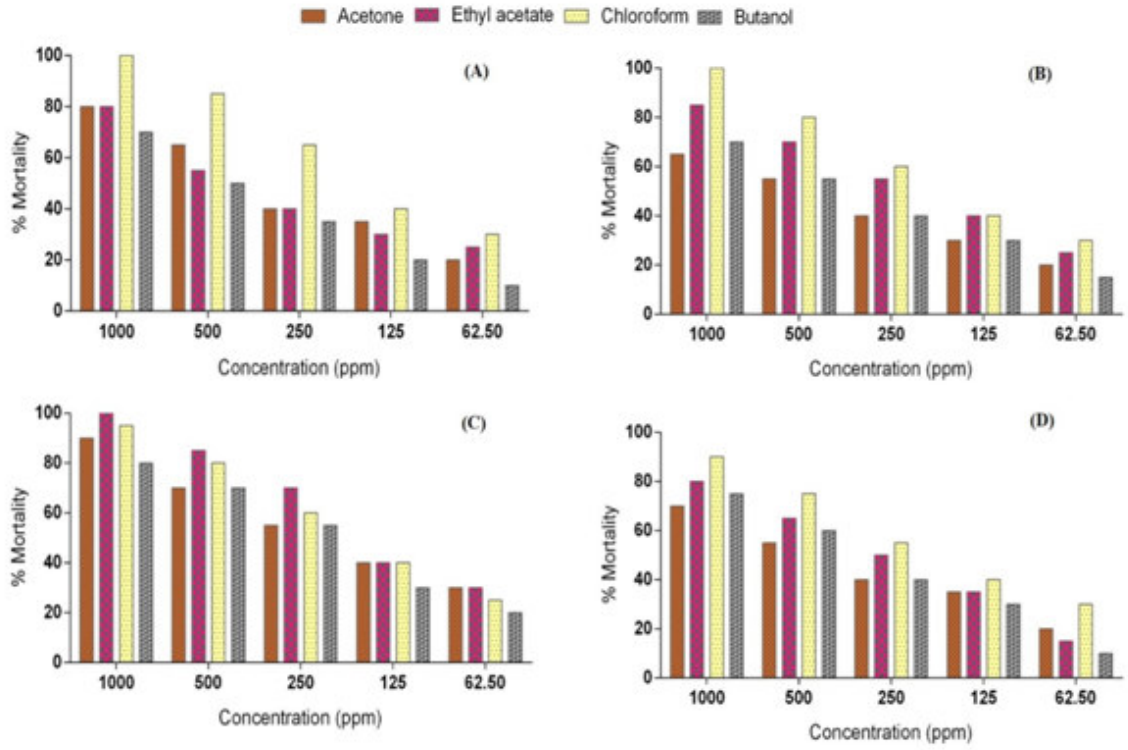

Table 1
Medicinal properties of tested for larvicidal activity

Plant name/family	Parts extracted	Vernacular name	Characteristic	Medicinal properties
Annona squamosa L./Annonaceae	Leaf	Seethapalam	Small tree	The leaf is insecticidal and is useful in destroying lice and as a poultice to produce suppuration. Seed paste is applied to the uterus of a pregnant woman to trigger abortion
Cynodon dactylon L/ Poaceae	Crushed *leaves*	Arugampul	Grass	The grass is sharp hot taste, good odor, laxative, brain and heart tonic, aphrodisiac, alexipharmic, emetic, emmenagogue, expectorant, carminative, pains, and inflammations
Melia azedarach L/ Meliaceae	Leaf	Malaivembu	Tree	Leaf, stem, root juice and paste are internally and externally used against stomachache, skin diseases and body pain
Hemidesmus indicus R. Br. /Apocynaceae	Root	*Nannari*	Twining shrub	The root is alterative tonic, demulcent, diaphoretic and traditionally been used to treat venereal diseases, skin diseases, urinary infections, arthritis and rheumatism

Table 2
Larvicidal activity of different solvent crude extracts against Culex quinquefasciatus

Plant	Solvent	LC_{50} ppm	UCL-LCL ppm
Annona squamosa	Acetone	594.60	788.50-448.39
	Ethyl acetate	490.35	654.08-367.60
	Chloroform	143.90	180.02-115.02
	Butanol	117.98	171.83-81.01
Cynodon dactylon	Acetone	585.86	758.34-452.60
	Ethyl acetate	143.32	192.11-106.92
	Chloroform	74.33	97.51-56.65
	Butanol	148.65	193.55-114.17
Melia azedarach	Acetone	264.87	325.01-215.85
	Ethyl acetate	65.27	98.04-43.45
	Chloroform	88.39	143.94-54.28
	Butanol	514.65	766.74-227.02
Hemidesmus indicus	Acetone	210.22	274.65-10.91
	Ethyl acetate	174.82	225.50-119.61
	Chloroform	117.98	171.83-81.01
	Butanol	445.45	467.87-311.07

Control—nil mortality. Significant at p<0.05 level,
LC_{50} lethal concentration that kills 50% of the exposed larvae,
UCL upperconfidence limit, LCL lower confidence limit

Table 3
Larvicidal activity of different solvent crude extracts against Aedes aegypti

Plant	Solvent	LC_{50} ppm	UCL-LCL ppm
Annona squamosa	Acetone	280.62	343.91-228.97
	Ethyl acetate	343.89	437.28-270.44
	Chloroform	156.92	195.92-125.69
	Butanol	471.94	594.01-374.95
Cynodon dactylon	Acetone	408.48	564.74-295.45
	Ethyl acetate	198.43	248.63-158.36
	Chloroform	169.28	215.16-133.19
	Butanol	385.55	503.43-295.27
Melia azedarach	Acetone	192.78	251.72-147.64
	Ethyl acetate	148.65	182.21-121.27
	Chloroform	172.24	210.55-140.90
	Butanol	235.97	288.14-193.24
Hemidesmus indicus	Acetone	363.91	484.11-273.56
	Ethyl acetate	255.47	311.69-209.39
	Chloroform	186.61	240.72-144.67
	Butanol	338.56	422.31-271.43

Control—nil mortality. Significant at p<0.05 level,
LC_{50} lethal concentration that kills 50% of the exposed larvae,
UCL upper confidence limit, LCL lower confidence limit

4. CONCLUSION

The environmentally benign and renewable source of ethyl acetate of *M. azedarach* extracts were highest mortality and control for mosquito larvae. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts. The isolation and purification of crude extract of leaf hexane and ethyl acetate of *M. azedarach* are in progress.

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