

Larvicidal activity of indigenous plant extracts on the rural malarial vector, *Anopheles culicifacies* Giles. (Diptera: Culicidae)

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Abstract

Vector control is one of the most important components in combating vector-borne diseases throughout the world. Application of insecticides is a widely known and popular vector control strategy. The objective of the present study was to evaluate the larvicidal activity of the hexane, diethyl ether, ethyl acetate and acetone extracts of *Abutilon indicum*, *Hyptis suaveolens* and *Leucas aspera* against third-stage larvae of *Anopheles culicifacies*. The results clearly suggest that all three selected plant extracts exhibited moderate larvicidal activity after 24, 48 and 72 h at 250, 500, 750 and 1000 ppm; the lethal concentrations (LC) at 50% and 90% of *A. indicum*, *H. suaveolens* against third instar larvae at 24, 48 and 72 h (hexane, diethyl ether, ethyl acetate and acetone) were as follows: *A. indicum*, LC₅₀=1031.65, 949.18, 833.58 and

673.68 ppm; LC₉₀=2215.87, 2234.39, 2152.97 and 2455.10 ppm; *H. suaveolens*, LC₅₀=423.00, 347.50, 236.58 and 217.24 ppm; LC₉₀=1431.91, 1292.15, 1138.49 and 1049.27 ppm and *L. aspera*, LC₅₀=559.77, 401.56, 299.71 and 263.01 ppm; LC₉₀=1400.80, 1549.31, 1157.96 and 1108.72 ppm at 24 h, respectively. Overall, the highest larvicidal activity was observed with *H. suaveolens* extract followed by *L. aspera* and *A. indicum* at various concentrations at 48 and 72 h, respectively. The objective of this investigation was an attempt to search for a user- and eco-friendly vector control agent. The study proved that the selected plant leaf extracts could serve as potent larvicidal agents against *A. culicifacies* in vector control programs.

Introduction

Malaria is a disease that inflicts a serious negative impact on public health and socio-economic development in resource-limited settings of the world. Malaria directly or indirectly affects the health and wealth of individuals as well as nations. Indeed, malaria is identified both as a disease associated with and a cause of poverty (Karunamoorthi, 2012). Currently, malaria control is hampered by many operational and technical problems. However, the development of insecticidal resistance in malaria vectors to existing conventional insecticides has made malaria vector control more challenging (Sharma & Saxena, 1996). *Anopheles stephensi* and *Anopheles culicifacies* are the two primary malarial vectors in India. In India, there are five sibling species reported, named A, B, C, D, and E; however, species A, C, D, and E are considered to be major vectors, while B is a minor vector (Subbarao *et al.*, 1999). Amerasinghe *et al.* (1999) reported that *A. culicifacies* is the main vector and *A. subpictus* is a significant secondary malarial vector in Sri Lanka. In India, *A. culicifacies* is a primary vector in rural as well as peri-urban areas, which constitutes nearly 65% of all malaria cases. At the moment, dichlorodiphenyltrichloroethane (DDT) (organochlorine), malathion (organophosphorus) and δ -methrin, cyfluthrin, α -cypermethrin and λ -cyhalothrin (synthetic pyrethroids) are the most commonly applied insecticides for vector control in the public health sector.

Insecticide susceptibility of *A. culicifacies* to DDT, malathion and methrin was evaluated in 2009 in several districts of India by adopting the WHO standard protocol for adult susceptibility (WHO, 1998). It was found that *A. culicifacies* has developed resistance to all insecticides tested (Mishra *et al.*, 2012). Currently, malaria control largely relies on a limited arsenal of materials; *viz.*, artemisinin derivative drugs and pyrethroids. However, these products could also become ineffective due to the continuing evolution of resistance development. In this context, a new innovative user- and eco-friendly alternative vector control strategy is mandatory. A search for powerful contextual community-based vector control interventions is therefore warranted.

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Abutilon indicum (Malvaceae), which is commonly known as Thuthi (the vernacular name in Tamil), is distributed throughout the driest areas in India (Chopra *et al.*, 1992). It is well known and reputable in the Tamil traditional medicinal system called Siddha as a phytotherapeutic agent against various illnesses such as jaundice, piles, ulcers and leprosy (Yoganarasimhan, 2000). It is also reported to possess effective analgesic activity; a study by Ahmed *et al.* (2000) indicated that an 80% ethanol root extract of *A. indicum* had a potential effect against *A. aegypti* and guppy fish (Promsiri *et al.*, 2006). The larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of *A. indicum*, *Aegle marmelos*, *Euphorbia thymifolia*, *Jatropha gossypifolia* and *Solanum torvum* were evaluated (Rahuman, 2008). Although *Hyptis suaveolens* is a native plant of tropical America, it is also widespread in tropical Africa, Asia and Australia. It grows under a wide range of soil and climatic conditions, mainly in warm areas of the country.

H. suaveolens is also administered as a traditional medicine for treatment of various ailments, and its essential oil possesses insecticidal and larvicidal properties (Peerzada, 1997; Azevedo *et al.*, 2001). In Nigeria, ethanolic extracts of orange peel (*Citrus sinensis*) and bush tea leaves of *H. suaveolens* were compared for their toxicity against *A. aegypti* (Amusan *et al.*, 2005). It has been reported that *H. suaveolens* extract causes notable mortality of *A. aegypti* larvae because the extract also contained insecticidal compounds such as a-terpinoline, a monoterpene that is similar in action to d-limonene, which is present in *C. sinensis*.

Leucas aspera (Labiatae) is a small herbaceous plant. It is commonly administered as an antipyretic herb in South India. The juice of its leaves is used for psoriasis and swellings as an external application. The plant extract mixed with honey is a good remedy for stomach pain and indigestion. Preliminary chemical examination of *L. aspera* revealed the presence of triterpenoids (Kamar & Singh, 1994). The whole plant is reported to contain oleanolic acid, ursolic acid, and 3-itosterol (Chaudhury & Ghosh, 1969). Aerial parts are reported to contain nicotine (Mangathayaru *et al.*, 2006). The flower is reported to contain ten compounds, among them amyl propionate (15.2%) and isoamyl propionate (14.4%), which were dominant (Kalachaveedu *et al.*, 2006). *L. aspera* leaves are used as an insecticide and mosquito repellent in rural areas (Kiritikar and Basu, 1990; Sadhu *et al.*, 2003). The hexane crude extracts of *L. aspera* showed high larvicidal activity against *C. quinquefasciatus* and *A. aegypti* (Maheswaran *et al.*, 2008; Kovendan *et al.*, 2012b). The aim of the present investigation was to determine the effect of *A. indicum*, *H. suaveolens* and *L. aspera* leaf extracts against third-stage larvae of *A. culicifacies* as a target species.

Materials and methods

Collection and rearing of mosquitoes

Larvae of *A. culicifacies* were collected from Kallar village, near Mettupalayam, Tamil Nadu, in different breeding habitats in using an *O* type brush. The mosquito larvae were fed with pedigree dog biscuits and yeast in a 3:1 ratio. Feeding was continued until the larvae transformed into the pupal stage. The pupae were collected from the culture trays using a dipper and transferred to plastic containers (12×12 cm) containing 500 mL of water. The plastic jars were kept in a 90×90×90-cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27±2°C, 75-85% relative humidity, under a photoperiod of 14:10 (light/dark). A 10% sugar solution was provided for a period of 3 days before blood feeding. The adult female mosquitoes were allowed to feed on the blood of a rabbit (one rabbit per day, exposed on the dorsal side) for 2 days to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Collection of plants and preparation of plant extracts

The selected medicinal plants were collected in and around Maruthamalai hills and Bharathiar University Campus, Coimbatore, Tamil Nadu. The fresh aerial part of *A. indicum*, and fresh leaves of *H. suaveolens* and *L. aspera* were washed thoroughly with tap water and shade dried at room temperature (28±2°C) for 5 to 12 days. The air-dried materials were powdered separately using a commercial electric blender. From each plant, 300 g of powdered material was macerated with 1.0 L of hexane, diethyl ether, ethyl acetate and acetone, sequentially, for a period of 72 h each and filtered. The yield of the *A. indicum*, *H. suaveolens* and *L. aspera* crude extracts with hexane, diethyl ether, ethyl acetate and acetone, were: *A. indicum* 8.94, 10.15, 9.36 and 11.55 g and *H. suaveolens* 8.12, 9.66, 10.47, and 9.73 g and *L. aspera* 11.12, 8.29, 9.34 and 10.13 g, respectively. The extracts were concentrated at a reduced temperature on a rotary vacuum evaporator and stored at a temperature of 4°C. One gram of the plant residue was dissolved in 100 mL of acetone, which was considered as a 1% stock solution, from which concentrations were prepared ranging from 250, 500, 750 and 1000 ppm, respectively.

Larval toxicity test

Larvicidal activity was assessed using the procedure of WHO (1996) with slight modifications. A laboratory colony of *A. culicifacies* larvae was used for the larvicidal activity. Twenty-five third-instar larvae of *A. culicifacies* were kept in 250-mL glass containers, containing 200 mL of dechlorinated water. Five replicates were set up for each concentration (250, 500, 750, 1000 ppm) and mixed with acetone and Triton-80 (mixing solution). Larval mortality was assessed at 24, 48 and 72 h. Each experiment was replicated 3 times at room temperature (28±2°C) for three different plants. The control mortalities were corrected by using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100 \quad (1)$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (2)$$

The lethal concentrations (LC) at 50% and 90% were calculated from toxicity data using probit analysis (Finney, 1971).

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC₅₀ and LC₉₀, and other statistics at a 95% upper fiducial limit and lower fiducial limit, and chi-square values were calculated using SPSS 9.0 version (Statistical software package; StataCorp., College Station, TX, USA). Results at P<0.05 were considered to be statistically significant.

Results

Preliminary screening is a good means of evaluating the potential larvicidal activity of crude plant extracts, which is often assessed using different solvent extracts. Mortality from the three plants tested is presented in Tables 1-3. At 24 h, *A. indicum* demonstrated mortality levels in hexane, diethyl ether, ethyl acetate and acetone extracts ranging from 22.18, 27.32, 32.55 and 41.23% at 24 h at 250 ppm to 51.65, 56.12, 61.55 and 64.35% at 1000 ppm, respectively (Table 1). For *H. suaveolens*, mortality levels at 24 h from these extracts ranged from 41.15, 46.33, 50.60 and 52.15% at 250 ppm, to 77.18, 83.14, 88.15 and 90.77% at 1000 ppm,

respectively (Table 2). Finally, for *L. aspera*, mortality at 24 h from these same extracts ranged from 31.12, 44.50, 46.66, and 49.15% at 250 ppm, to 74.18, 75.27, 87.55 and 89.26% at 1000 ppm, respectively (Table 3). We observed correspondingly higher larval mortality from these extracts at higher concentrations after 48 and 72 h, respectively (Tables 2 and 3).

The LC₅₀ and LC₉₀ values of hexane, diethyl ether, ethyl acetate and acetone extracts of *A. indicum*, *H. suaveolens* and *L. aspera* at 24 were as follows: *A. indicum*, LC₅₀=1031.65, 949.18, 833.58 and 673.68 ppm; and LC₉₀=2215.87, 2234.39, 2152.97 and 2455.10 ppm, respectively. For *H. suaveolens*, LC₅₀=423.00, 347.50, 236.58 and 217.24 ppm; and LC₉₀=1431.91, 1292.15, 1138.49 and 1049.27 ppm, respectively. Finally, for *L. aspera*, LC₅₀=559.77, 401.56, 299.71 and 263.01 ppm; and LC₉₀=1400.80, 1549.31, 1157.96 and 1108.72 ppm at 24 h, respectively (Tables 1-3).

Discussion

A. culicifacies is one of the major malaria vectors in the Indian sub-continent and is generally regarded as intolerant to salinity, preferring to breed in newly-dug freshwater pits, domestic wells and sites used for plantings of coconuts and casurina trees in India (Russell & Rao, 1942; Sabesan *et al.*, 1986). Ansari *et al.* (2000) suggested that the peppermint oil (*Mentha piperita*) showed strong repellent action against adult mosquitoes when applied on human skin. The protection obtained against *A. annularis*, *A. culicifacies*, and *C. quinquefasciatus* was 100, 92.3, and 84.5%, respectively. The present investigation tested three plant leaf extracts in different solvents for their potential larvicidal

activity against *A. culicifacies*. The biological activity of the experimental plant extracts varied, which may be due to the presence of various phytochemically active compounds in the plants, including phenolics, terpenoids, flavonoids and alkaloids. These active principles may have jointly or independently influenced or contributed to produce larvicidal effects against *A. culicifacies*.

Sharma & Ansari (1994) demonstrated the protective effect of *Cyperus rotundus* against *A. culicifacies*, *A. stephensi* and *C. quinquefasciatus*, and showed greater protection than neem oil (37.5%). Sharma *et al.* (1995), reported that the crude extract of *Solanum nigrum* leaves showed significant larvicidal activity against *A. culicifacies*, *C. quinquefasciatus* and *A. aegypti* at a dose equivalent to the LC₉₀, ranging from 0.18 to 0.21% (Singh *et al.*, 2002). The present results concur with some of the previous findings of *A. indicum* against the third instar larvae of *A. culicifacies*, with LC₅₀ and LC₉₀ values of hexane, diethyl ether, ethyl acetate and acetone extracts of (LC₅₀) 1031.65, 949.18, 833.58 and 673.68 ppm, and (LC₉₀) 2215.87, 2234.39, 2152.97 and 2455.10 ppm at 24 h, respectively.

One study reported that the lethal concentration values of the aqueous extract of roots of *H. abelmoschus* against the larvae of *A. culicifacies*, *A. stephensi*, and *C. quinquefasciatus* were 52.3, 52.6, and 43.8 ppm, respectively (Dua *et al.*, 2006). The LC₅₀ and LC₉₀ values of *S. indicus*, *C. collinus* and *M. koenigii* against third-instar larvae at 24, 48 and 72 h (in hexane, chloroform and ethyl acetate extracts) were: for *S. indicus*, (LC₅₀) 544.93, 377.86 and 274.79 ppm, and (LC₉₀) 1325.32, 1572.55 and 1081.29 ppm at 24 h; for *C. collinus*, (LC₅₀) 375.34, 318.29 and 226.10 ppm, and (LC₉₀) 699.65, 1577.62 and 1024.92 ppm at 24 h; and, for *M. koenigii*, (LC₅₀) 963.53, 924.85 and 857.62 ppm, and (LC₉₀) 1665.12, 1624.68 and 1564.37 ppm at 24 h, respectively (Kovendan *et al.*, 2012a).

Table 1. Larvicidal activity of *Abutilon indicum* against third instars larvae of *Anopheles culicifacies*.

Exposure (time)	Solvents	Percentage larval mortality±SD Concentration of <i>A. indicum</i> (ppm)				LC ₅₀ (LC ₉₀)	95% confidence limit		x ² (df=4)
		250	500	750	1000		LFL LC ₅₀ (LC ₉₀)	UFL LC ₅₀ (LC ₉₀)	
24 h	Hexane	22.18±0.86	26.31±1.12	34.56±0.98	51.65±1.50	1031.65 (2215.87)	877.34 (1733.59)	1360.29 (3414.23)	1.39 ^a
	Diethyl ether	27.32±0.42	30.40±0.65	37.19±1.32	56.12±1.25	949.18 (2234.39)	802.18 (1722.60)	1260.00 (3594.44)	2.41 ^a
	Ethyl acetate	32.55±1.23	34.12±1.45	40.66±0.44	61.55±1.18	833.58 (2152.97)	700.82 (1659.17)	1076.37 (3484.70)	3.78 ^a
	Acetone	41.23±1.65	43.65±0.68	45.22±1.36	64.35±1.33	673.68 (2455.10)	479.50 (1742.72)	928.05 (5435.70)	3.52 ^a
48 h	Hexane	30.63±0.54	38.50±1.69	55.60±1.74	68.92±1.95	655.69 (1586.80)	561.41 (1333.98)	757.04 (2079.68)	0.56 ^a
	Diethyl ether	34.12±1.55	42.26±0.72	58.77±0.95	71.23±1.27	592.82 (1556.39)	487.55 (1303.21)	690.49 (2061.01)	0.48 ^a
	Ethyl acetate	44.35±1.21	49.22±1.56	61.10±1.22	74.88±1.48	444.90 (1625.26)	255.91 (1310.21)	562.52 (2373.37)	1.10 ^a
	Acetone	50.56±1.35	63.91±1.75	71.25±1.66	85.45±1.30	274.54 (1207.15)	69.32 (1026.23)	392.03 (1561.63)	0.76 ^a
72 h	Hexane	48.61±1.52	65.22±1.88	84.14±1.47	91.35±1.32	273.83 (935.20)	140.65 (831.15)	362.35 (1100.14)	0.46 ^a
	Diethyl ether	51.70±0.80	70.50±1.95	91.36±1.93	96.50±1.40	245.95 (764.39)	136.20 (688.68)	321.82 (873.81)	0.99 ^a
	Ethyl acetate	58.44±1.65	79.20±1.20	93.18±1.65	98.42±1.91	230.45 (676.51)	130.83 (611.13)	299.81 (767.98)	0.14 ^a
	Acetone	61.12±1.40	80.10±1.33	96.54±1.21	100.00±0.00	179.87 (594.49)	66.42 (532.79)	253.45 (681.76)	1.95 ^a

Control, nil mortality; SD, standard deviation; LC₅₀, LC₉₀, lethal concentration at 50% and 90%; LFL, lower fiducial limit; UFL, upper fiducial limit; x², Chi-square value; df, degrees of freedom. Mean values of five replicates. ^aSignificant at P<0.05 level.

Table 2. Larvicidal activity of *Hyptis suaveolens* against the third instars larvae of *Anopheles culicifacies*.

Exposure (time)	Solvents	Percentage larval mortality±SD Concentration of <i>H. suaveolens</i> (ppm)				LC ₅₀ (LC ₉₀)	95% confidence limit		x ² (df=4)
		250	500	750	1000		LFL LC ₅₀ (LC ₉₀)	UFL LC ₅₀ (LC ₉₀)	
24 h	Hexane	41.15±0.94	54.59±1.23	65.20±1.13	77.18±1.19	423.00 (1431.91)	262.74 (1195.44)	527.13 (1920.40)	0.06 ^a
	Diethyl ether	46.33±1.89	57.16±0.88	68.15±0.92	83.14±1.93	347.50 (1292.15)	171.86 (1094.04)	454.55 (1682.95)	0.72 ^a
	Ethyl acetate	50.60±1.51	67.24±0.85	72.13±1.80	88.15±1.37	236.58 (1138.49)	22.09 (973.62)	357.75 (1454.65)	1.83 ^a
	Acetone	52.15±0.88	69.10±1.46	74.78±1.69	90.77±0.98	217.24 (1049.27)	15.04 (907.98)	334.37 (1305.85)	2.00 ^a
48 h	Hexane	43.18±0.56	58.14±0.84	68.93±0.92	79.50±1.25	367.14 (1343.81)	191.69 (1130.97)	474.69 (1772.94)	0.08 ^a
	Diethyl ether	48.50±1.16	63.20±0.91	70.18±0.79	86.00±1.77	284.56 (1207.01)	86.93 (1027.61)	399.26 (1555.88)	1.19 ^a
	Ethyl acetate	51.65±1.27	68.15±1.85	75.21±1.54	89.14±0.69	219.38 (1081.48)	9.19 (931.45)	339.44 (1360.06)	1.00 ^a
	Acetone	54.01±0.85	70.36±0.78	79.36±1.10	92.56±0.75	196.53 (962.17)	4.99 (841.05)	309.95 (1170.61)	0.91 ^a
72 h	Hexane	47.10±1.27	65.48±1.35	77.23±1.16	84.78±1.68	268.78 (1134.84)	80.81 (977.06)	380.42 (1426.91)	0.51 ^a
	Diethyl ether	57.03±0.78	79.85±1.18	85.55±0.77	98.32±0.88	164.33 (751.50)	7.30 (668.80)	262.41 (875.67)	3.56 ^a
	Ethyl acetate	70.01±0.96	97.36±0.89	100.0±0.00	100.0±0.00	158.80 (382.51)	56.706 (339.78)	211.84 (452.69)	0.04 ^a
	Acetone	75.13±0.70	98.56±0.00	100.00±0.00	100.00±0.00	138.30 (349.48)	6.89 (307.90)	195.95 (421.39)	0.01 ^a

Control, nil mortality; SD, standard deviation; LC₅₀, LC₉₀, lethal concentration at 50% and 90%; LFL, lower fiducial limit; UFL, upper fiducial limit; x², Chi-square value; df, degrees of freedom. Mean values of five replicates. ^aSignificant at P<0.05 level.

Table 3. Larvicidal activity of *Leucas aspera* against third instars larvae of *Anopheles culicifacies*.

Exposure (time)	Solvents	Percentage larval mortality±SD Concentration of <i>L. aspera</i> (ppm)				LC ₅₀ (LC ₉₀)	95% confidence limit		x ² (df=4)
		250	500	750	1000		LFL LC ₅₀ (LC ₉₀)	UFL LC ₅₀ (LC ₉₀)	
24 h	Hexane	31.12±1.17	47.12±1.45	62.14±1.88	74.18±1.25	559.77 (1400.80)	464.21 (1203.49)	643.56 (1758.85)	0.09 ^a
	Diethyl ether	44.50±1.20	52.43±1.94	65.36±1.70	75.27±1.33	401.56 (1549.31)	198.85 (1259.51)	520.40 (2219.35)	0.22 ^a
	Ethyl acetate	46.66±1.32	65.16±1.80	69.52±1.57	87.55±0.88	299.71 (1157.96)	126.24 (996.97)	405.43 (1455.63)	2.48 ^a
	Acetone	49.15±1.50	66.98±1.57	71.55±1.36	89.26±1.44	263.01 (1108.72)	77.46 (956.98)	373.42 (1387.02)	2.56 ^a
48 h	Hexane	37.92±0.95	54.35±1.65	68.12±1.30	77.10±1.65	426.33 (1377.58)	279.40 (1163.25)	524.96 (1800.20)	0.14 ^a
	Diethyl ether	45.42±0.77	61.72±1.10	73.50±1.55	82.64±1.93	312.92 (1223.97)	131.45 (1044.38)	421.96 (1568.28)	0.16 ^a
	Ethyl acetate	48.16±1.13	69.41±1.72	76.92±1.42	89.18±1.18	253.69 (1038.54)	81.55 (906.06)	359.15 (1269.33)	0.95 ^a
	Acetone	51.75±1.95	78.56±1.23	87.30±1.22	93.48±1.35	183.68 (824.85)	22.88 (732.59)	284.62 (967.34)	2.14 ^a
72 h	Hexane	45.60±1.26	61.54±0.90	73.81±1.18	87.36±1.44	325.57 (1119.70)	178.16 (974.76)	420.93 (1374.18)	0.38 ^a
	Diethyl ether	52.76±1.17	79.30±1.29	91.10±1.94	94.75±1.20	183.77 (761.60)	39.44 (680.09)	276.83 (881.77)	2.18 ^a
	Ethyl acetate	63.15±0.92	86.25±1.12	96.63±1.61	100.00±0.00	144.51 (560.83)	14.56 (499.23)	225.14 (646.87)	0.63 ^a
	Acetone	88.80±1.13	93.24±1.50	100.00±0.00	100.00±0.00	138.24 (478.13)	11.798 (423.46)	211.30 (557.32)	2.54 ^a

Control, nil mortality; SD, standard deviation; LC₅₀, LC₉₀, lethal concentration at 50% and 90%; LFL, lower fiducial limit; UFL, upper fiducial limit; x², Chi-square value; df, degrees of freedom. Mean values of five replicates. ^aSignificant at P<0.05 level.

It is known that placing *H. suaveolens* branches or whole plants in and around houses is one of the most effective methods in western Kenya, for repelling the malaria vector *Anopheles gambiae* Giles (Seyoum *et al.*, 2002). Kovendan *et al.* (2012b) reported on the effects of hexane, chloroform, ethyl acetate and methanol extracts of *J. curcas* against third instar larvae of *C. quinquefasciatus*, with LC₅₀ values of 230.32, 212.85, 192.07 and 113.23 ppm, respectively. For *H. suaveolens*, LC₅₀ values of these extracts were 213.09, 217.64, 167.59 and 86.93 ppm, respectively. For *A. indicum*, the LC₅₀ values were 204.18, 155.53, 166.32 and 111.58 ppm, respectively. Finally, for *L. aspera*, LC₅₀ values were 152.18, 118.29, 111.43 and 107.73 ppm, respectively. Similarly, our results with *H. suaveolens* against the third-instar larvae of *A. culicifacies* showed LC₅₀ and LC₉₀ values of hexane, diethyl ether, ethyl acetate and acetone extracts of (LC₅₀) 423.00, 347.50, 236.58 and 217.24 ppm, and (LC₉₀) 1,431.91, 1292.15, 1138.49 and 1049.27 ppm at 24 h, respectively.

Previous studies have been conducted using a methanol extract of *Clerodendron inerme* and *Acanthus ilicifolius* at different concentrations (20, 40, 60, 80 and 100 ppm), with LC₅₀ values against *A. stephensi* first to fourth-instar larvae and pupae of 55.04, 63.33, 73.05, 80.74 and 74.33 ppm, and 52.76, 57.76, 63.36, 70.18 and 62.78 ppm, respectively. Corresponding LC₉₀ values were 125.50, 137.16, 153.55, 156.93, 199.20 ppm, and 108.30, 115.83, 125.24, 131.28 and 141.03 ppm, respectively (Kovendan & Murugan, 2011). Mahesh Kumar *et al.* (2012) reported the LC₅₀ and LC₉₀ values of *S. xanthocarpum* against the first to fourth instar larvae and pupae of *C. quinquefasciatus* as 155.29, 198.32, 271.12, 377.44, and 448.41 ppm, and 687.14, 913.10, 1,011.89, 1,058.85, and 1,141.65 ppm, respectively. In the present results, the hexane, diethyl ether, ethyl acetate and acetone extracts of *L. aspera* against third-instar larvae of *A. culicifacies* had LC₅₀ values of 559.77, 401.56, 299.71 and 263.01 ppm, and LC₉₀ values of 1400.80, 1549.31, 1157.96 and 1108.72 ppm at 24 h, respectively.

Conclusions

The larvicidal properties of crude extracts of *A. indicum*, *H. suaveolens* and *L. aspera* against *A. culicifacies* were studied under laboratory conditions. The results clearly demonstrated the highest mortality with *H. suaveolens*, followed by *L. aspera* and *A. indicum*. In addition, the study also showed that the solvents used for the extractions have some impact on the level of larval mortality. The highest mortality was seen with acetone extract followed by ethyl acetate, diethyl ether and hexane. This mortality profile demonstrates the extraction properties of different solvents from which the maximum effect was obtained. We conclude that botanicals could be a better alternative than relying on the most hazardous currently used synthetic chemical insecticides and bio-pesticides, and could contribute to a healthier environment. The use of plant-based products could be an ideal eco- and user-friendly vector control strategy for diminishing and eventual elimination of the malaria burden in the near future.

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