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Larvicidal efficacy of *Cassia fistula* Linn. leaf extract against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera:Culicidae)

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ABSTRACT

Objective: To determine the larvicidal efficacy of different solvent leaf extract of *Cassia fistula* (*C. fistula*) against *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*) and *Anopheles subpictus* (*An. subpictus*). **Methods:** Twenty five early third instar of *Cx. tritaeniorhynchus* and *An. subpictus* were exposed to various concentrations and were assayed in the laboratory by using the protocol of WHO 2005. The larval mortality was observed after 24 h of treatment. **Results:** Among three solvent extracts tested the maximum efficacy was observed in the methanol extract. The LC_{50} and LC_{90} values of *C. fistula* against early third instar of *Cx. tritaeniorhynchus* and *An. subpictus* were 45.57, 33.76 ppm and 82.05, 60.63 ppm, respectively. No mortality was observed in controls. The *Chi*-square values were significant at $P< 0.05$ level. **Conclusions:** From the results it can be concluded the crude extract of *C. fistula* has an excellent potential for controlling *Cx. tritaeniorhynchus* and *An. subpictus* mosquito larvae.

1. Introduction

Vector and vector-borne diseases have become a challenging problem to public health in these days as it has social and economical impact especially in subtropical and tropical countries. Mosquitoes are the most important arthropod disease vectors, transmitting nine dreadful human diseases over 100 countries, causing mortality of nearly two million people every year[1]. The mosquito control, therefore, continues to be an important strategy in preventing the mosquito-borne diseases[2]. Control of mosquito during their development in aquatic medium by employing a very effective method is highly warranted for the sake of public health. The extensive and indiscriminate application of synthetic chemical insecticides lead to environmental and health concerns, widespread development of resistance by mosquitoes and unwarranted toxic or lethal effects on non-target organisms. These well known drawbacks with synthetic insecticides shifted the mosquito control programme to the use of eco-friendly, bio-degradable and microbial plant compounds with mosquitocidal property. Pushpanathan *et al*[3] stated that essential oils from *Cymbopogan citratus* (*C. citratus*) were evaluated for larvicidal, ovicidal and repellent activities against the filarial mosquito *Culex quinquefasciatus* (*Cx. quinquefasciatus*). The methanolic leaf extract of *Cassia fistula* (*C. fistula*) was tested for larvicidal and ovicidal activity against *Cx. quinquefasciatus* and *Anopheles stephensi* (*An. stephensi*)[4]. Larvicidal efficacy of the leaf extract of *Citrullus colocynthis* (*C. colocynthis*) with four different solvents like ethyl acetate, benzene, petroleum ether and methanol were tested against the late third instar larvae of *An. stephensi*, *Cx. quinquefasciatus*, and *Aedes aegypti* (*Ae. aegypti*)[5]. Essential oils from *Zingiber officinalis* was evaluated for larvicidal and repellent activity against the filarial mosquito *Cx. quinquefasciatus*[6].

The leaf extract of *Acalypha indica* (*A. indica*) with different solvents *viz.*, benzene, chloroform, ethyl acetate and methanol were tested for larvicidal, ovicidal activity and oviposition attractancy against *An. stephensi*[7]. The leaf extract of *C. fistula* with different solvens *viz.*, methanol, benzene and acetone were studied for the larvicidal, ovicidal and repellent activity against *Ae. aegypti*[8]. Samidurai *et al*[9] observed that the leaf extracts of *Pemphis acidula* (*P. acidula*) were evaluated for larvicidal, ovicidal and repellent activities against *Cx. quinquefasciatus* and *Ae. aegypti*. Govindarajan[10] have investigated the larvicidal efficacy of different extracts of *Ficus benghalensis* (*F. benghalensis*) L. against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. Mathivanan *et al*[11] have determined the larvicidal efficacy of *Eria coronaria* (*E. coronaria*) leaves extract against

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Cx. quinquefasciatus, *Ae. aegypti* and *An. stephensi*. The larvicidal and repellent activities of *Sida acuta* (*S. acuta*) and *Clausena anisata* extract against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were assayed[12,13]. The larvicidal activity of *S. acuta* was evaluated against 3rd instar larvae of *Anopheles subpictus* (*An. subpictus*) and *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*)[14].

Govindarajan and Karupannan have reported the larvicidal and ovicidal activities of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Eclipta alba* (*E. alba*) against dengue vector, *Ae. aegypti*[15]. The larvicidal and ovicidal efficacy of different extracts of *Cardiospermum halicacabum* (*C. halicacabum*) L. against *Cx. quinquefasciatus* and *Ae. aegypti* were studied[16]. The larvicidal and ovicidal activity of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of the leaves of three plants, *E. alba* (*E. alba*), *C. halicacabum*, and *Andrographis paniculata* (*A. paniculata*), were tested against the early third-instar larvae of *An. stephensi*[17]. The larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogan citrates*, *Cinnamomum zeylanicum*, *Rosmarinus officinalis* and *Zingiber officinale* against *Cx. tritaeniorhynchus* and *An. subpictus* were evaluated[18]. The larvicidal, ovicidal, and repellent activities of crude benzene and ethyl acetate extracts of leaf of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were assayed for their toxicity against three important vector mosquitoes, *viz.*, *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*[19]. The larvicidal and ovicidal efficacy of different extracts of *A. paniculata* against *Cx. quinquefasciatus* and *Ae. aegypti*[20]. The larvicidal efficacy of different solvent leaf extract of *Ficus benghalensis* (*F. benghalensis*) against *Cx. tritaeniorhynchus* and *An. subpictus* were tested[21]. *C. fistula* L. (Leguminosae) a semi-wild Indian Labernum (also known as the Golden shower) is distributed in various parts throughout the world including Asia, South Africa, Mexico, China, West Indies, East Africa, and Brazil. The plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections[22]. The whole plant is used to treat diarrhea; seeds are used to treat skin diseases; flowers and fruits are used to treat skin diseases, fever, abdominal pain, and leprosy. This plant extract possesses pest and disease control capacities. The present study is aimed to test the larvicidal efficacy of different solvent leaf extracts of *C. fistula* against *An. subpictus* and *Cx. tritaeniorhynchus*.

2. Materials and methods

2.1. Plant collection

Fully developed leaves of the *C. fistula* were collected from different regions of Cuddalore District, Tamilnadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University.

2.2. Preparation of the extract

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant material (3.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with three different solvents namely benzene, acetone, and methanol individually. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. Standard stock solutions were prepared at 1% by dissolving the residues in appropriate solvent. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassay.

2.3. Test organisms

Cx. tritaeniorhynchus and *An. subpictus* were reared in the Vector Control Laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28 ± 2) ℃, 70 % - 85 % relative humidity (RH), with a photoperiod of 14 h light, 10 h dark.

2.4. Larvicidal bioassay

The Larvicidal activity of the plant crude extracts was evaluated as per the method recommended by WHO[23]. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 mL of water. The appropriate volume of dilution was added to 200 mL water in the cups to obtain the desired target dosage (concentration ranging from 20 to 100 ppm), starting with the lowest concentration. six replicates were set up for each concentration and an equal number of controls were set up simultaneously using tap water. To this 1 mL of appropriate solvent was added. The LC_{50} value was calculated after 24 h by probit analysis[24].

2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit. *Chi*-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The efficacy of *C. fistula* was tested against the early third larvae of *Cx. tritaeniorhynchus* and *An. subpictus*. The data were recorded and statistical data regarding the LC_{50}, LC_{90}, *Chi*-square and 95% confidence limits were calculated (Table 1). The methanolic extract of *C. fistula* showed highest larvicidal activity against *Cx. tritaeniorhynchus* and *An. subpictus*. The LC_{50} values of methanol, benzene and acetone extracts of *C. fistula* against early third larvae of *Cx. tritaeniorhynchus* and *An. subpictus* were 45.57 ppm, 48.55 ppm, 52.17 ppm and 33.76 ppm, 36.43 ppm, 39.01 ppm, respectively. No mortality was observed in control. The *chi*-square value were significant at $P< 0.05$ level.

Table 1
Larvicidal activity of different solvent leaf extracts of *C. fistula* against *Cx. tritaeniorhynchus* (CT), *An. subpictus* (AS).

Solvents	Concentration (ppm)		% of mortality ± SD		LC_{50} (LCL-UCL) (ppm)		LC_{90} (LCL-UCL) (ppm)		χ^2	
	CT	AS	CT	AS	CT	AS	CT	AS	CT	AS
Methanol	Control	Control	0.0 ± 0.0	0.0 ± 0.0	45.57 (33.69-56.86)	33.76 (25.23-41.87)	82.05 (68.47-108.77)	60.63 (50.83-79.44)	17.666*	16.728*
	20	15	25.8 ± 0.6	26.2 ± 1.4						
	40	30	48.7 ± 1.2	48.5 ± 1.2						
	60	45	65.3 ± 1.0	66.2 ± 1.0						
	80	60	82.9 ± 0.8	84.4 ± 1.2						
	100	75	99.8 ± 1.4	99.9 ± 0.8						
Benzene	Control	Control	0.0 ± 0.0	0.0 ± 0.0	48.55 (37.58-59.30)	36.43 (29.47-43.34)	84.68 (71.55-109.64)	62.43 (53.75-77.57)	16.097*	12.746*
	20	15	20.3 ± 1.0	22.3 ± 1.8						
	40	30	46.2 ± 1.6	38.6 ± 1.4						
	60	45	61.3 ± 1.2	69.3 ± 1.4						
	80	60	80.4 ± 1.6	84.5 ± 1.2						
	100	75	99.6 ± 0.8	99.6 ± 1.0						
Acetone	Control	Control	0.0 ± 0.0	0.0 ± 0.0	52.17 (42.19-62.26)	39.01 (32.42-45.69)	89.15 (76.41-112.27)	66.32 (57.65-81.02)	13.679*	11.115*
	20	15	16.2 ± 1.4	19.6 ± 1.0						
	40	30	42.1 ± 0.8	35.2 ± 1.2						
	60	45	57.4 ± 1.2	57.8 ± 1.4						
	80	60	76.2 ± 1.6	79.3 ± 0.8						
	100	75	98.2 ± 1.0	98.2 ± 1.4						

*- Significant at $P<0.05$ level. LC_{50}- Lethal concentration; LCL- Lower confidence limit; UCL- Upper confidence limit.

4. Discussion

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. Botanical extracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties. Our result showed that the crude benzene, acetone and methanol extracts of leaf of *C. fistula* have significant larvicidal properties against two important vector mosquitoes *viz.*, *An. subpictus* and *Cx. tritaeniorhynchus*. This result is also comparable to earlier reports of Govindarajan[8], in whose reports the leaf extract of *C. fistula* with different solvents *viz.*, methanol, benzene and acetone were studied for the larvicidal activity against *Ae. aegypti*. The 24 h LC_{50} concentration of the extract against *Ae. aegypti* were observed at 10.69, 18.27 and 23.95 mg/L respectively. Pushpanathan *et al*[3] stated that essential oils from *C. citratus* were evaluated for larvicidal activity against the filarial mosquito *Cx. quinquefasciatus*. The LC_{50} values calculated for the 2nd, 3rd and 4th larval instar were (144.54 ± 2.3) ppm, (165.70 ± 1.2) ppm and (184.18 ± 0.8) ppm respectively. Methanolic leaf extract of *C. fistula* was found to be more lethal to the larvae of *An. stephensi* than *Cx. quinquefasciatus* with LC_{50} values of 17.97 mg/L and 20.57 mg/L, respectively[4]. The leaf extract of *A. indica* with different solvents *viz.*, benzene, chloroform, ethyl acetate and methanol were tested for larvicidal activity against *An. stephensi*. The LC_{50} values are 19.25, 27.76, 23.26 and 15.03 ppm, respectively[7]. The LC_{50} values of methanol, benzene, acetone extract of *P. acidula* against *Cx. quinquefasciatus* and *Ae. aegypti* were 10.81 ppm, 41.07 ppm, 53.22 ppm and 22.10 ppm, 43.99 ppm, 57.66 ppm, respectively[9]. The LC_{50} values of *F. benghalensis* against early second, third and fourth larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were 41.43, 58.21 and 74.32 ppm, 56.54 , 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm respectively[10].

Mathivanan *et al*[11] have determined the LC_{50} and LC_{90} values of crude methanol extract of leaves of *E. coronaria* on *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* larvae in 24 h, and the results were 72.41 and 65.67 mg/L, 62.08 and 136.55 mg/L, 127.24 and 120.86 mg/L, respectively. Govindarajan[12] evaluated larvicidal activity of crude extract of *S. acuta* against three important mosquitoes with LC_{50} values ranging between 38 and 48 mg/L. The essential oil from the leaves of *C. anisata* exhibited significant larvicidal activity, with 24 h LC_{50} values of 140.96, 130.19 and 119.59 ppm, respectively[13]. The larvicidal activity of *S. acuta* was evaluated against 3rd instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*. The leaf extract and active compound cryptolepine showed negligible mortality against early third instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*. The 24h LC_{50} value was observed at 38.68, 50.81mg/L and 9.98, 12.69mg/L for crude leaf extract and active compound cryptolepine, respectively[14]. The LC_{50} values of benzene, hexane, ethyl acetate, methanol and chloroform extract of *E. alba* against early third instar larvae of *Ae. aegypti* were 151.38, 165.10, 154.88, 127.64 and 146.28 ppm, respectively[15]. The larvicidal efficacy of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Cardiospermum halicacabum* (*C. halicacabum*) against *Cx. quinquefasciatus* and *Ae. aegypti*. The LC_{50} values were 174.24, 193.31, 183.36, 150.44, 154.95 ppm and 182.51, 200.02, 192.31, 156.80, 164.54 ppm, respectively[16].

The larvicidal activity of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of the leaf of three plants, *E. alba*, *C. halicacabum*, and *A. paniculata* against *An. stephensi* was investigated. The highest larval mortality was found in methanol extract of *A. paniculata*, *E. alba*, and *C. halicacabum* against the larvae of *An. stephensi* (LC_{50} = 79.68, 112.56, and 133.01 ppm; LC_{90} = 154.66, 220.68, and 270.72 ppm)[17]. The highest larvicidal activity was observed in the essential oil from *Z. officinale* against *Cx. tritaeniorhynchus* and *An. subpictus* with the LC_{50} and LC_{90} values at 98.83, 57.98 ppm and 186.55, 104.23 ppm,

respectively[18]. The larvicidal activity of crude benzene and ethyl acetate extracts of leaf of *E. coronaria* and *C. pulcherrima* against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* was tested. The highest larval mortality was found in benzene extract of *E. coronaria* against the larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* with the LC_{50} and LC_{90} values at 79.08, 89.59 ppm, 96.15, 150.47 ppm, 166.04, 174.10 ppm, respectively[19]. The benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *A. paniculata* was found to be more effective against *Cx. quinquefasciatus* than *Ae. aegypti*. The LC_{50} values against this two species were 112.19, 137.48, 118.67, 102.05, 91.20 ppm and 119.58, 146.34, 124.24, 110.12, 99.54 ppm respectively[20]. The LC_{50} and LC_{90} values of methanol extract of *F. benghalensis* against early third instar of *Cx. tritaeniorhynchus* and *An. subpictus* were 100.88, 159.76 ppm and 56.66, 85.84 ppm, respectively[21]. Compared with earlier authors' reports and our earlier reports, the present results revealed that the experimental plant extracts were effective to control *An. subpictus* and *Cx. tritaeniorhynchus*. From these results it could be concluded that the plant *C. fistula* exhibits larvicidal activity against two important vector mosquitoes. Further analysis to isolate the active compound for larval control is under way in our laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

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