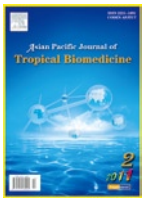
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

Larvicidal efficacy of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae)

Arivoli S[1], Samuel T[2*]

[1]*P.G. & Research Department of Advanced Zoology and Biotechnology Loyola College, Chennai 600 034, Tamilnadu, India*
[2]*Department of Zoology, Madras Christian College, Chennai 600 059, Tamilnadu, India*

ARTICLE INFO

Article history:
Received 15 August 2011
Received in revised form 2 September 2011
Accepted 28 September 2011
Available online 15 October 2011

Keywords:
Cleistanthus collinus leaf extracts
Aedes aegypti
Anopheles stephensi
Culex quinquefasciatus
Larvicidal

ABSTRACT

Objective: To determine the larvicidal activity of *Cleistanthus collinus* (*C. collinus*) leaf extracts against *Aedes aegypti*, *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus*. **Methods:** The larvicidal activity was determined against three vector mosquito species at concentrations of 250, 500, 750 and 1000 ppm. Larval mortality was assessed after 24 hours. **Results:** The leaf extracts of *C. collinus* was found to exhibit a larvicidal activity against the larvae of *An. stephensi* with a LC_{50} value of 399.72 ppm. **Conclusions:** The results indicate moderate level of larvicidal activity against vector mosquitoes.

1. Introduction

Mosquitoes represent a significant threat to human health because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide[1]. Several species belonging to genera *Aedes*, *Anopheles* and *Culex* are vectors for the pathogens of various diseases like dengue fever, dengue haemorrhagic fever, malaria, Japanese encepahalitis and filariasis[2-4]. Over and injudicious use of synthetic insecticides in vector control has resulted in environmental hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non-target organisms[5,6]. Plant derived materials are comparatively safer to humans and ecosystem and easily biodegradable[7]. Plant derived natural products have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic insecticides[8]. Phytochemicals extracted from various plant species have been tested for their actions against mosquitoes[9].

Cleistanthus collinus (*C. collinus*) (Roxb.) is a toxic plant belonging to the family Euphorbiaceae and it grows in the dry forests of southern and central parts of India, Malaysia and Africa. It is commonly called as "garari" in Hindi, "oduvan" in Tamil, "vadise" in Telugu and "nilapala" in Malayalam[10]. Many parts of the plant were reported as toxic and the aqueous extract of the crushed leaves of this plant are used as cattle and fish poison, abortifacient, suicidal and homicidal agents. The alcoholic extract of the leaves, roots and fruits of *C. collinus* are used to treat gastro intestinal disorders and it also possess anticancer activity. Further, the plant also possesses insecticidal properties against the red flour beetle, *Tribolium castaneum* and are used as insecticides in rice fields[10,11]. The leaf extracts of this plant exhibited insecticidal properties such as antifeedant and insect growth regulatory against the larvae of *Spodoptera litura*[12-14]. Therefore the prescent study was carried out to determine the larvicidal activity of *C. colliuns* leaf extracts against against vector mosquitoes.

2. Materials and methods

2.1 Plant collection and extraction

C. collinus leaves collected in and around Tamilnadu, India were brought to the laboratory, shade dried under room temperature and powdered using an electric blender. A total of 1 kg of dried and powdered leaves was subjected

*Corresponding author: Dr. Samuel Tennyson, Assistant Professor, Department of Zoology, Madras Christian College, Chennai 600 059, Tamilnadu, India.
E-mail: samtennyson@gmail.com
Foundation Project: This work was financially supported by Department of Science and Technology, Goverment of India (grant No. SR/FT/L-96/2005).

to sequential extraction using 3 L of hexane, diethyl ether, dichloromethane and ethyl acetate for a period of 72 h to obtain the crude extracts using rotary vacuum evaporator. The hexane, diethyl ether, dichloromethane and ethyl acetate crude extracts thus obtained were lyophilized and a stock solution of 100 000 ppm prepared from each crude extract by adding adequate volume of acetone was refrigerated at 4 ℃ until testing for bioassays.

2.2. Test mosquitoes

All tests were carried out against laboratory reared vector mosquitoes *viz.*, *Aedes aegypti* (*Ae. aegypti*), *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) free of exposure to insecticides and pathogens. Cyclic generations of vector mosquitoes were maintained at 25–29 ℃ and 80–90 % relative humidity in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio of 3:1) and adult mosquitoes on 10 % glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

2.3. Larvicidal activity

Standard WHO protocol with slight modifications was adopted for the study[15]. From the stock solution, concentrations of 250, 500, 750 and 1 000 ppm were prepared. Twenty five early third instar larvae were introduced in 250 mL beaker containing 200 mL of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 24 hours. A total of three trials were carried out with five replicates per trial against vector mosquitoes. However, when the control mortality ranged from 5–20 per cent, the observed percentage mortality was corrected by Abbott's formula[16],

$$\text{Per cent mortality} = \frac{\%\ \text{Mortality in treated} - \%\ \text{Mortality in control}}{100 - \%\ \text{Mortality in control}} \times 100$$

2.4. Statistical analysis

SPSS 11.5 version package was used for determination of LC_{50} and LC_{90}[17]. Data from mortality and effect of concentrations were subjected to analysis of variance. The percentage data obtained was angular transformed. Difference between the treatments was determined by Tukey's test ($P < 0.05$).

3. Results and discussion

Results of the larvicidal effects of leaf extracts of *C. collinus* against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* reported in the present study exhibit the mosquitocidal properties in the plant suggesting their use in mosquito population control (Table 1, 2). The leaf extracts of *C. collinus* showed larval mortality. *An. stephensi* was more susceptible followed by *Ae. aegypti* and *Cx. quinquefasciatus*. The ethyl acetate extract of *C. collinus* exhibited the maximum larvicidal activity with LC_{50} value of 399.72 ppm against the larvae of *An. stephensi*. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices. The results of present study are comparable with earlier reports. Sharma *et al*[18] reported that the petroleum ether extract of *Ageratum conyzoides* leaves exhibited larvicidal activity with LC_{50} value of 425.60 and 267.90 ppm after 24 and 48 h of exposure. The toxicity to the third instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *Memordica charantia*, *Trichosanthus anguina* and *Luffa acutangula* showed the LC_{50} values of 465.85, 567.81 and 839.81 ppm respectively[19]. The toxicity to the late third instar larvae of *Ae. aegypti* by the hexane leaf extracts of *Abutilon indicum* and *Cx. quinquefasciatus* by dichloromethane whole plant extracts of *Citrullus colocynthis* and hexane extracts of aerial parts of *Hyptis suaveolens* was reported by Arivoli and Samuel[20-22]. The findings of the present investigation revealed that the leaf extracts of *C. collinus* possess larvicidal activity against vector mosquitoes. It may concluded that natural products as extracts from parts of plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity. Further studies on the screening, isolation and purification

Table 1
Larvicidal activity of *C. collinus* leaf extracts against vector mosquitoes.

Mosquito spp.	Solvents	Mortality rate (%) (Mean ± SD)			
		250 ppm	500 ppm	750 ppm	1 000 ppm
Ae. aegypti	Hexane	5.60 ± 3.58 (13.7)^{a}	18.40 ± 3.58 (25.4)^{b}	30.40 ± 4.56 (33.5)^{b}	39.20 ± 5.22 (38.8)^{b}
	Diethyl ether	13.60 ± 3.58 (21.6)^{b}	34.40 ± 7.80 (35.9)^{c}	35.20 ± 5.93 (36.4)^{b}	61.60 ± 4.56 (51.7)^{c}
	Dichloromethane	14.40 ± 3.58 (22.3)^{b}	37.60 ± 5.37 (37.8)^{c}	34.40 ± 6.69 (35.9)^{b}	64.80 ± 3.35 (53.6)^{cd}
	Ethyl acetate	34.40 ± 4.56 (35.9)^{c}	29.60 ± 4.56 (33.0)^{c}	62.40 ± 6.07 (52.2)^{c}	70.40 ± 6.07 (57.0)^{d}
	Control	0^{a}	0^{a}	0^{a}	0^{a}
An. stephensi	Hexane	4.80 ± 3.35 (12.7)^{a}	19.20 ± 3.35 (26.0)^{b}	29.60 ± 4.56 (32.9)^{b}	34.40 ± 6.07 (35.9)^{b}
	Diethyl ether	2.40 ± 3.58 (8.9)^{a}	4.80 ± 3.35 (12.7)^{a}	5.60 ± 5.37 (13.7)^{a}	7.20 ± 7.16 (15.6)^{a}
	Dichloromethane	18.40 ± 3.58 (25.4)^{b}	29.60 ± 4.56 (32.9)^{c}	33.60 ± 3.58 (35.4)^{b}	72.80 ± 5.22 (58.6)^{c}
	Ethyl acetate	39.20 ± 5.22 (38.8)^{c}	43.20 ± 3.35 (41.1)^{d}	64.80 ± 7.69 (53.6)^{c}	100.00 ± 0.00 (90.0)^{d}
	Control	0^{a}	0^{a}	0^{a}	0^{a}
Cx. quinquefasciatus	Hexane	5.60 ± 3.58 (13.7)^{ab}	8.80 ± 5.22 (17.3)^{b}	33.60 ± 3.58 (35.4)^{c}	39.20 ± 5.22 (38.8)^{b}
	Diethyl ether	2.40 ± 3.58 (8.9)^{a}	6.40 ± 2.19 (14.7)^{ab}	17.60 ± 6.69 (24.8)^{b}	36.80 ± 8.20 (37.4)^{b}
	Dichloromethane	3.20 ± 4.38 (10.3)^{a}	4.80 ± 3.35 (12.7)^{ab}	16.80 ± 1.79 (24.2)^{b}	30.40 ± 6.69 (33.5)^{b}
	Ethyl acetate	13.60 ± 6.69 (21.6)^{b}	38.40 ± 6.69 (38.3)^{c}	44.80 ± 4.38 (42.0)^{d}	61.60 ± 3.58 (51.7)^{c}
	Control	0^{a}	0^{a}	0^{a}	0^{a}

Figures in parentheses are angular transformed. ANOVA followed by TUKEY test performed. Different superscripts in the column indicate significance difference at $P < 0.05$ levels.

Table 2
Probit analysis of larvicidal efficacy of *C. collinus* leaf extracts against vector mosquitoes.

Mosquito spp.	Extracts	LC_{50} (ppm) 24 h	LC_{90} (ppm) 24 h	*Chi*-square value	Regression value
Ae. aegypti	Hexane	1291.21	5070.99	0.03*	2.16
	Diethyl ether	837.36	3450.87	4.98*	2.08
	Dichloromethane	755.26	2831.15	3.71*	2.23
	Ethyl acetate	560.41	2669.86	7.10	1.89
An. stephensi	Hexane	1398.29	5833.61	0.82*	2.07
	Diethyl ether	602.04	5451.98	0.43*	1.12
	Dichloromethane	776.13	3066.50	18.76	2.15
	Ethyl acetate	399.72	1251.76	39.92	2.58
Cx. quinquefasciatus	Hexane	3253.94	5697.99	5.90*	2.50
	Diethyl ether	1434.66	4047.84	3.60*	2.84
	Dichloromethane	1808.10	6418.15	3.71*	2.33
	Ethyl acetate	755.75	2962.00	1.81*	2.16

* Significant at $P < 0.05$ level.

of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *C. collinus* leaf extracts to control the immature stages of vector mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are thankful to the Department of Science and Technology, New Delhi, Govt. of India for financial assistance.

References

[1] WHO. Malaria fact sheets No. 94. WHO Report. Genava: WHO media center; 2010.
[2] Borah R, Kalita MC, Kar A, Talukdar AK. Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus*. *African J Biotechnol* 2010; **9**(16): 2527-2530.
[3] Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res* 2009; **104**: 1365-1372.
[4] Samuel T. Studies on the mosquitocidal activity of *Ageratum houstonianum* Mill. (Asteraceae) against vector mosquitoes [Phd Thesis]. Tamilnadu, India: University of Madras; 2010.
[5] Bansal SK, Singh KV, Sharma S, Sherwani MRK. Comparative larvicidal potential of different plant parts of *Withania somnifera* against vector mosquitoes in the semi-arid region of Rajasthan. *J Environ Biol* 2011; **32**(1): 71-75.
[6] Devine GJ, Furlong MJ. Insecticide use: Contexts and ecological successions. *Agr Human Values* 2007; **24**: 281-306.
[7] Kalu IJ, Ofoegbu U, Eroegbusi J, Nwachukwu CU, Ibeh B. Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. *J Med Plant Res* 2010; **4**(6): 496-498.
[8] Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chaithong U, Kanjanapothi R, et al. Aromatic plant-derived essential oil: An alternative larvicide for mosquito control. *Fitoterapia* 2007; **78**: 205-210.
[9] Pavela R. Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *Parasitol Res* 2008; **102**: 555-559.
[10] Harwansh RK, Dangi JS, Jha AK, Deshmukh R. Effect of medicinal plant Garari (*Cleistanthus collinus*) Family: Euphorbiaceae against red flour beetles (*Tribolium castaneum*). *J Pharm Res* 2010; **3**(5): 965-968.
[11] Gupta R, Vairale MG, Deshmukh RR, Chaudhary PR, Wate SR. Ethnomedicinal uses of some plants used by Gond tribe of Bhandara district, Maharashtra. *Indian J Tradit Knowledge* 2010; **9**(4): 713-717.
[12] Selvamuthukumaran T, Arivudainambi S. Insect growth regulatory action of certain leaf fractions of *Cleistanthus collinus* (Roxb.) Benth (Family: Euphorbiaceae) against *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). *Hexapoda* 2008; **15**(2): 125-127.
[13] Selvamuthukumaran T, Arivudainambi S. Insecticidal properties of *Cleistanthus collinus* (Roxb.) Benth (Family: Euphorbiaceae) against *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). *Plant Arch* 2008; **8**(2): 683-685.
[14] Selvamuthukumaran T, Arivudainambi S. Dose dependant differential anti insect activity of lactone glycoside, a potent plant derived molecule. *J Biopest* 2010; **3**(1): 259-264.
[15] WHO. Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES/IC/96. 1. Geneva: Control of Tropical Diseases Division; 1996.
[16] Abbott WS. A method of computing the effectiveness of an insecticide. *J Eco Entomol* 1925; **18**: 265-267.
[17] SPSS. SPSS for windows, version 11.5. Chicago, IL: SPSS; 2007.
[18] Sharma P, Mohan L, Srivastava CN. Anti-juvenile activity of *Azadirachta indica* extract on the development and morphometry of filaria vector, *Culex quinquefasciatus* (Diptera: Culicidae) Say. *Parasitol Res* 2009; **105**: 1193-1203.
[19] Prabakar K, Jebanesan A. Larvicidal efficacy of some cucurbitaceous plant leaf extracts against *Culex quinquefasciatus* (Say). *Bioresour Technol* 2004; **95**: 113-114.
[20] Arivoli S, Samuel T. Larvicidal and adult emergence inhibition of *Abutilon indicum* (Linn.) (Malvaceae) leaf extracts against vector mosquitoes. *J Biopest* 2011; **4**(1): 27-35.
[21] Arivoli S, Samuel T. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) whole plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Int J Curr Res* 2011; **3**(4): 296-304.
[22] Arivoli S, Samuel T. Mosquitocidal activity of *Hyptis suaveolens* (L) Poit (Lamiaceae) extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). *Int J Rec Sci Res* 2011; **2**(5): 143 -149.