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Larvicidal activity of endocarp and seed crude extracts of *Dracaena loureiri* Gagnep against *Aedes aegypti* (L.) mosquito

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ABSTRACT

Objective: To evaluate the larvicidal activity of the ethanolic and aqueous extracts of the endocarp and seeds of *Dracaena loureiri* (*D. loureiri*) against the dengue mosquito vector, *Aedes aegypti*.

Methods: Bioassays were performed by exposing late third-stage to early fourth-stage larvae of *Aedes aegypti* to various concentrations of the extracts from *D. loureiri*. The larval mortality was observed after 24- and 48-h exposure.

Results: The larvicidal bioassay in this study demonstrated that the ethanolic endocarp extract was the most effective with the LC_{50} value of 84.00 mg/L after 24 h exposure and < 50 mg/L after 48 h exposure. Extracts from the other parts of the plant were significantly less effective as a larvicide.

Conclusions: The ethanolic endocarp extract of *D. loureiri* demonstrated effective larvicidal activity. It is an alternative source for developing a novel larvicide for controlling this mosquito species.

1. Introduction

Insecticide has long been used for controlling insect vectors, especially when an outbreak of vector-borne disease has occurred. To control the outbreak of dengue and/or dengue hemorrhagic fevers, several insecticides have been used for reducing the population of *Aedes aegypti* (*Ae. aegypti*), the biological vector of the dengue virus. Temephos is the leading chemical insecticide which has been applied to household water containers to control *Ae. aegypti* larvae [1]. As well, while temephos is highly effective against the mosquito larvae, it has a low toxicity for humans. However, it has been reported that continuous uptake may have negative effects on humans. Also, inappropriate use of this insecticide can result in insecticide resistance in the vector [2]. Currently, plant based bio-insecticides are intensively studied worldwide, especially in tropical countries with a high diversity of plants. Particular attention has been paid to the study on many plant extracts for determining their larvicidal potential against *Ae. aegypti*. In some countries, the abundance of such plants has led to the study and development of what may be termed "folkloric medicine", or natural remedies derived from plants.

One Thai folkloric medicine is based on *Dracaena loureiri* Gagnep (*D. loureiri*), with the common name of "*Chan Pha*", "*Chan Daeng*" or "*Lukka Chan*". *D. loureiri* belongs to the family of Asparagaceae. It has long been used as an antipyretic and an analgesic for the treatment of cough, fever and inflammation. Previous studies indicated that crude extracts of *D. loureiri* stem wood have anti-allergic and estrogenic properties, as well as anti-HIV-1 reverse transcriptase and antimalarial (*Plasmodium falciparum*) properties [3–6]. Although some biological activities of *D. loureiri* were reported, larvicidal activity against any mosquito vector has not been found in the

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literature. Moreover, the fruit (endocarp and seed) of *D. loureiri* has not been extracted and studied.

The purpose of this study was, therefore, to evaluate the larvicidal activity of ethanolic and aqueous extracts of *D. loureiri* endocarp and seed against the *Ae. aegypti* mosquito.

2. Materials and methods

2.1. D. loureiri fruit extracts

Fresh fruits of *D. loureiri* were collected on October 30, 2013 from naturally growing trees in Phitsanulok Province, Thailand. Some of the *D. loureiri* fruits were kept as voucher specimens (DTNU008) and deposited at the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Thailand. The fruits were cleaned with tap water. Then, the endocarps and seeds were separated and weighed. The endocarps (359.70 g) and seeds (393.42 g) were dried in a hot air oven at 45 °C until completely dried. They were then ground using an electric blender at 22 000 r/min. The dried powder of the endocarps (79.40 g) and seeds (192.25 g) were extracted with absolute ethanol and distilled water in a ratio of 1:10 (powder: solvent, w/v). Twenty-five grams of the powder were suspended in 250 mL of the solvent in 500-mL Erlenmeyer flasks, which were continuously shaken at 180 r/min on a rotary shaker for 24 h at room temperature. The suspension was then suction filtered through a Whatman No. 1 filter paper via a Buchner funnel. The filtrates of ethanolic extract were concentrated by using a rotary evaporator (Büchi Rotavapor® R-205 with Büchi Vac® V-500, Büchi, Switzerland), while the aqueous extracts were concentrated by using the same evaporation protocol, and then dried by using a lyophilizer (Lyotrap LF/LYO/01/1, LTE Scientific, UK). Yields for the ethanolic crude extract of the endocarps and seeds were 3.76 and 4.23 g, respectively. The aqueous extracts of endocarps and seeds yielded 40.69 and 11.21 g, respectively. All crude extracts were kept in a desiccator until required for a further bioassay.

2.2. Ae. aegypti mosquito colonization

Aedes spp. larvae were obtained from several breeding containers in Muang District, Phitsanulok Province, Thailand, and transported to the laboratory in the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Thailand. They were reared in tap water under laboratory conditions [(25 ± 2) °C, 70%–80% relative humidity, and 10:14 (light: dark) photoperiod]. The larvae were fed with powdery dog biscuits. After pupation, they were transferred into plastic cups filled with tap water and covered with a net until they became adults. The 2–3 days old adults were individually identified according to their morphology following the illustrated keys to the mosquitoes in Thailand [7]. The mosquitoes identified as *Ae. aegypti* were transferred into a mosquito cage (30 cm × 30 cm × 30 cm). They were provided with 5% sugar mixed with 5% multivitamin syrup solution. After 5 days, the females were allowed to feed on a blood meal by using an artificial membrane-feeding method. After further 3–4 days, the gravid female mosquitoes were permitted to lay eggs on a wet filter paper (Whatman No. 1). The eggs were air-dried and maintained in a humidity-controlling glass jar until used. A colony of *Ae. aegypti* was established for mass producing of larvae for the larvicidal bioassay.

2.3. Larvicidal bioassay

The larvicidal activity of the *D. loureiri* extracts was tested against the *Ae. aegypti* larvae by following the protocol of World Health Organization [8]. Briefly, a stock solution of aqueous extracts (1%, w/v) was prepared by adding 200 mg of the extract and 20 mL of distilled water. For the preparation of a stock solution of ethanolic extracts, dimethylsulphoxide was used as a diluent (1%, w/v). The stock solutions were kept in a refrigerator at 4 °C. A series of concentrations were prepared for testing the larvicidal activity, and 200 mL of each concentration of each extract was put into plastic bowls. Twenty-five of the third stage larvae were transferred into the crude extract solutions. For both seed and endocarp aqueous extracts and seed ethanolic extract, the concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1 000 mg/L were prepared. For the endocarp ethanolic extract, the concentrations of 50, 100, 150, 200, 250, and 300 mg/L were prepared. After 24 and 48 h, mortality rates were determined by using a needle. The larvae were considered dead when they were unable to normally move after gentle touching. The experiments were performed in four replicates, with 100 larvae for each concentration of each crude extract. Two control groups, one containing 200 mL of distilled water only, and one of 2 mL of dimethylsulphoxide in 198 mL distilled water, were used for the testing for aqueous and ethanolic extract, respectively.

2.4. Data analysis

The mortality data from the larval bioassay were analyzed using a computerized probit analysis for the LC_{50} value determination [9]. The *Chi*-square values and 95% fiducial confidence intervals [upper and lower limits (UL and LL)] were also calculated. The computerized program is a commercial LdP Line® software (Plant Protection Research Institute, Egypt).

3. Results

The results for larvicidal activities of ethanolic and aqueous crude extracts of *D. loureiri* fruits against the 3rd stage larvae of *Ae. aegypti* after 24- and 48-h exposure are presented in Tables 1 and 2, respectively.

After 24-h exposure, the ethanolic extract of endocarp showed the highest activity with LC_{50} value of 84.00 mg/L, while the seed ethanol and endocarp aqueous extracts showed much higher LC_{50} values of 921.69 and 1 067.53 mg/L, respectively. After 48-h exposure, the larval mortality rate shown by the ethanolic endocarp extract was very high with > 90% mortality exhibited by the lowest concentration (50 mg/L). The LC_{50} value of the ethanolic endocarp extract could not be calculated with the Ldp Line software, so the LC_{50} value was estimated to be < 50 mg/L for this extract. The LC_{50} values after 48-h exposure were < 50, 307.40 and 834.37 mg/L for the endocarp ethanolic, seed ethanolic and endocarp aqueous extracts, respectively. For the seed aqueous extract, no larval mortality was detected after 24 h, and only a very low larval

Table 1

Larvicidal activities of crude ethanolic and aqueous *D. loureiri* extracts against the 3rd stage *Ae. aegypti* larvae after 24-h exposure.

Crude extract (mg/L)		Mortality (mean ± SE) (%)	Larvicidal activity		
			LC_{50} (UL–LL) (mg/L)	χ^2	Slope ± SE
Endocarp ethanol	50	29.00 ± 2.52	84.00 (71.02–95.85)	8.70	2.28 ± 0.22
	100	64.00 ± 4.32			
	150	69.00 ± 1.91			
	200	72.00 ± 4.32			
	250	88.00 ± 3.65			
	300	93.00 ± 1.00			
	Control	0			
Endocarp aqueous	100	0	1 067.53[a] (960.52–1 241.93)	2.55	3.11 ± 0.35
	200	0			
	300	5.00 ± 3.79			
	400	10.00 ± 2.58			
	500	12.00 ± 3.65			
	600	24.00 ± 6.32			
	700	25.00 ± 5.26			
	800	38.00 ± 7.75			
	900	39.00 ± 3.42			
	1 000	48.00 ± 5.66			
	Control	0			
Seed ethanol	100	0	921.69 (848.00–1 029.11)	12.13	3.24 ± 0.33
	200	0			
	300	6.00 ± 1.15			
	400	11.00 ± 1.91			
	500	27.00 ± 2.52			
	600	29.00 ± 3.79			
	700	35.00 ± 3.42			
	800	35.00 ± 4.43			
	900	42.00 ± 4.16			
	1 000	65.00 ± 4.12			
	Control	1.00 ± 1.00			
Seed aqueous	100	0	–[b]	–[b]	–[b]
	200	0			
	300	0			
	400	0			
	500	0			
	600	0			
	700	0			
	800	0			
	900	0			
	1 000	0			
	Control	0			

[a] : The LC_{50} value is estimated by the probit analysis of Ldp Line software; [b] : No mortality rates were observed from all concentrations, so the parameters could not be calculated.

Table 2

Larvicidal activities of crude ethanolic and aqueous *D. loureiri* extracts against the 3rd stage *Ae. aegypti* larvae after 48-h exposure.

Crude extract (mg/L)		% Mortality (mean ± SE)	Larvicidal activity		
			LC_{50} (UL–LL) (mg/L)	χ^2	Slope ± SE
Endocarp ethanol	50	93.00 ± 1.00	< 50[a]	–[b]	–[b]
	100	94.00 ± 1.15			
	150	96.00 ± 1.63			
	200	99.00 ± 1.00			
	250	100			
	300	100			
	Control	0			
Endocarp aqueous	100	0	834.37 (777.62–908.25)	8.46	3.42 ± 0.29
	200	1.00 ± 1.00			
	300	5.00 ± 3.79			
	400	19.00 ± 4.12			
	500	17.00 ± 5.97			
	600	36.00 ± 6.93			
	700	36.00 ± 8.49			
	800	54.00 ± 4.76			
	900	52.00 ± 7.12			
	1 000	58.00 ± 9.59			
	Control	0			
Seed ethanol	100	3.00 ± 1.00	307.40 (289.07–325.40)	9.04	5.19 ± 0.38
	200	21.00 ± 4.43			
	300	47.00 ± 6.61			
	400	63.00 ± 5.51			
	500	90.00 ± 4.76			
	600	97.00 ± 1.91			
	700	100			
	800	100			
	900	100			
	1 000	100			
	Control	2.00 ± 1.15			
Seed aqueous	100	0	–[c]	–[c]	–[c]
	200	0			
	300	0			
	400	0			
	500	0			
	600	0			
	700	0			
	800	3.00 ± 1.91			
	900	17.00 ± 1.91			
	1 000	27.00 ± 3.00			
	Control	0			

[a] : The mortality rates were very high, so the accurate LC_{50} could not be calculated; [b] : The mortality rates were very high, so the parameters could not be calculated; [c] : The mortality rates were very low, so the parameters could not be calculated.

mortality rate was found after 48 h. This demonstrates that seed aqueous extract is not effective as a larvicide against the *Ae. aegypti* mosquito larvae.

The LC_{50} values of the crude extracts that contained larvicide (except the 48 h ethanolic endocarp extract) were compared and statistically analyzed. The results, shown in Figure 1, were that the ethanolic endocarp extract exhibited the highest larvicidal activity after 24 h among the other extracts with LC_{50} value of 84.00 mg/L, followed by the ethanolic seed extract after

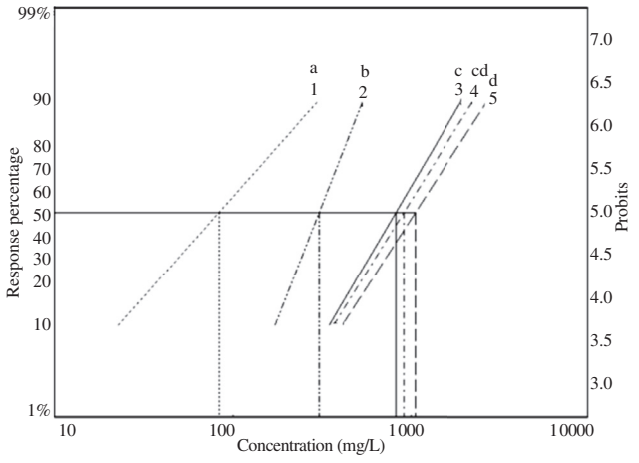

Figure 1. Graph showing the LC_{50} values of crude ethanolic and aqueous *D. loureiri* extracts against the 3rd stage *Ae. aegypti* larvae after 24- and 48-h exposure.
1: Endocarp ethanol (24 h); 2: Seed ethanol (48 h); 3: Endocarp aqueous (48 h); 4: Seed ethanol (24 h); 5: Endocarp aqueous (24 h). Endocarp ethanol (48 h) and seed aqueous (both 24 h and 48 h) extracts are excluded, because of the very high and very low observed mortality rates, respectively. Statistically significant differences are indicated by different letters on the crude extract categories.

48 h (307.40 mg/L). The aqueous encocarp (24 and 48 h) and the seed ethanol (24 h) extracts revealed significantly lower activities.

4. Discussion

This study identified a new and promising property, the larvicidal activity against *Ae. aegypti* mosquito, of the *D. loureiri* extracts. The aqueous extracts of *D. loureiri* did not show any activity. However, the ethanol extracts, especially for the endocarp, revealed a strong larvicidal efficacy with LC_{50} value of 84.00 mg/L after 24-h exposure. The comparable efficacy against the same mosquito larva, *Ae. aegypti*, was also found in other studies. Ethanolic extracts of *Garcinia mangostana* crown showed the larvicidal efficacy with the LC_{50} value of 63.00 mg/L [10]. For the other solvents, acetone extract of *Basella rubra* leaf and benzene extract of *Cleome viscosa* leaf showed the LC_{50} values of 72.63 and 82.43 mg/L, respectively against the *Ae. aegypti* larvae [11]. Chloroform and methanol leaf extracts of *Erythrina indica* showed the LC_{50} values of 95.62 and 75.13 mg/L, respectively [12]. Recently, ethyl acetate extract of *Chloroxylon swietenia* leaf demonstrated larvicidal activity with LC_{50} value of 80.58 mg/L [13].

For the literature regarding Asparagaceae extracts, insecticidal activity of this plant family has only recently been reported in the study of Govindarajan and Sivakumar [14]. The ovicidal, larvicidal and adulticidal properties of root extracts of *Asparagus racemosus* (Willd.) (*A. racemosus*) against *Ae. aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* mosquitoes have also been reported. The methanol extract of the *A. racemosus* root revealed an effective larvicidal activity against *Ae. aegypti* with the LC_{50} value of 97.71 mg/L after 24-h exposure. When compared with the methanol extract of *A. racemosus* root, the ethanolic extract of *D. loureiri* endocarp had stronger efficiency with 84.00 mg/L LC_{50} value found at the same exposure time. Higher activity was found (LC_{50} value < 50 mg/L) after leaving the mosquito larvae with the extract solution for 48 h. The similar finding for the better larvicidal activity of 48-h exposure was generally reported. Ethanolic crude extract of *Cnidoscolus phyllacanthus* showed LC_{50} value of 0.246 mg/L after 48-h exposure compared to the LC_{50} value of 1.103 mg/L after 24-h exposure [15]. Recently, similar findings have been reported for *Pereskia bleo* endocarp crude extract, with LC_{50} values of 416.50 (48 h) and 1094.84 mg/L (24 h), respectively [16]. Also, the 48-h larvicidal activities (LC_{50} values of 263.90, 300.80 and 342.20 mg/L) of *Coriandrum sativum*, *Nigella sativa* and *Syzygium aromaticum* ether extracts against *Aedes albopictus* were higher than the 24-h larvicidal activity (LC_{50} values of 363.70, 377.50 and 403.40 mg/L) [17].

Because the literature regarding *D. loureiri* activity against arthropods is limited, the action causing larval death could not be compared in this study. However, a sodium chloride extract of *D. loureiri* was tested for a colchicine-like property against adult *Ae. aegypti* [18]. *D. loureiri* did not reveal a promising result when compared to *Gloriosa superba* (*G. superba*) extract which showed the best colchicine-like activity. Colchicine effectively inhibits the cell division via disruptive action on the microtubule polymerization causing the mitosis to be discontinued [19]. In mosquitoes, colchicine has the same effect in both adult and larval stages; therefore colchicine-like substance may be assumed to be a cause of larval death in this study. Recently, acetone extract of *G. superba* was found to kill 3rd and 4th instar *Ae. aegypti* larvae with the LC_{50} values of 34.62 and 40.47 mg/L, respectively [20]. We expected that the larvicidal activity of *D. loureiri* might come from the same action as the *G. superba* did, *i.e.* the colchicine-like property. It is suggested that the actual action of *D. loureiri* extract against the mosquito larva should be further investigated. Furthermore, Govindarajan and Sivakumar [14] indicated the larvicidal activity along with ovicial and adulticidal activities from the member of Asparagaceae family, *A. racemosus*. Therefore, ovicidal and adulticidal activity may be discovered from the *D. loureiri* extracts and should be evaluated further.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] George L, Lenhart A, Toledo J, Lazaro A, Han WW, Velayudhan R, et al. Community-effectiveness of temephos for dengue vector control: a systematic literature review. *PLoS Negl Trop Dis* 2015; **9**(9): e0004006.

[2] Grisales N, Poupardin R, Gomez S, Fonseca-Gonzalez I, Ranson H, Lenhart A. Temephos resistance in *Aedes aegypti* in Colombia compromises dengue vector control. *PLoS Negl Trop Dis* 2013; **7**(9): e2438.

[3] Makchuchit S, Itharat A, Tewtrakul S. Anti-allergic activity of Thai medicinal plants. *Planta Med* 2009; http://dx.doi.org/10.1055/s-0029-1234527.

[4] El-Halawany AM, El Dine RS, Chung MH, Nishihara T, Hattori M. Screening for estrogenic and antiestrogenic activities of plants growing in Egypt and Thailand. *Pharmacogn Res* 2011; **3**(2): 107-13.

[5] Silprasit K, Seetaha S, Pongsanarakul P, Hannongbua S, Choowongkomon K. Anti-HIV-1 reverse transcriptase activities of hexane extracts from some Asian medicinal plants. *J Med Plants Res* 2011; **5**(19): 4899-906.

[6] Thiengsusuk A, Chaijaroenkul W, Na-Bangchang K. Antimalarial activities of medicinal plants and herbal formulations used in Thai traditional medicine. *Parasitol Res* 2013; **112**(4): 1475-81.

[7] Rattanarithikul R, Harbach RE, Harrison BA, Panthusiri P, Coleman RE, Richardson JH. Illustrated keys to the mosquitoes of Thailand. VI. Tribe Aedini. *Southeast Asian J Trop Med Public Health* 2010; **41**(Suppl 1): 1-225.

[8] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005. [Online] Available from: http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf [Accessed on 24th November]

[9] Finney DJ. *Probit analysis*. 3rd ed. Cambridge: Cambridge University Press; 1971, p. 1-333.

[10] Torres RC, Garbo AG, Walde RZML. Larvicidal activity of *Garcinia mangostana* fruit wastes against dengue vector *Aedes aegypti*. *J Anim Plant Sci* 2015; **25**(4): 1187-90.

[11] Krishnappa K, Elumalai K. Mosquitocidal properties of *Basella rubra* and *Cleome viscosa* against *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci* 2013; **17**: 1273-7.

[12] Govindarajan M, Sivakumar R. Larvicidal, ovicidal, and adulticidal efficacy of *Erythrina indica* (Lam.) (Family: Fabaceae) against *Anopheles stephensi, Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2014; **113**: 777-91.

[13] Jayaraman M, Senthikumar A, Venkatesalu V. Evaluation of some aromatic plant extracts for mosquito larvicidal potential against *Culex quinquefasciatus, Aedes aegypti*, and *Anopheles stephensi*. *Parasitol Res* 2015; **114**: 1511-8.

[14] Govindarajan M, Sivakumar R. Ovicidal, larvicidal and adulticidal properties of *Asparagus racemosus* (Willd.) (Family: Asparagaceae) root extracts against filariasis (*Culex quinquefasciatus*), dengue (*Aedes aegypti*) and malaria (*Anopheles stephensi*) vector mosquitoes (Diptera: Culicidae). *Parasitol Res* 2014; **113**(4): 1435-49.

[15] Candido LP, Cavalcanti MT, Beserra EB. Bioactivity of plant extracts on the larval and pupal stages of *Aedes aegypti* (Diptera, Culicidea). *Rev Soc Bras Med Trop* 2013; **46**(4): 420-5.

[16] Thongwat D, Ganranoo L, Chokchaisiri R. Larvicidal activity of *Pereskia bleo* (Kunth) DC. (Cactaceae) fruit endocarp crude and fractionated extracts against *Aedes aegypti* (L.) (Diptera: Culicidae). *Southeast Asian J Trop Med Public Health* 2014; **45**(6): 1292-300.

[17] Bilal H, Akram W, Din S, Khan IA, Hassan SA, Arshad M. Larvicidal activity of selected plant extracts against *Aedes albopictus* Skuse (Diptera: Culicidae). *Afr Entomol* 2012; **20**(1): 8-12.

[18] Jitpakdi A, Choochote W, Insun D, Tippawakgkosol P, Keha P, Pitasawat B. Screening of ten plant species for metaphase chromosome preparation in adult mosquitoes (Diptera: Culicidae) using and inoculation technique. *J Med Entomol* 1999; **36**(6): 892-5.

[19] Haraguchi T, Kaneda T, Hiraoka Y. Dynamics of chromosomes and microtubules visualized by multiple-wavelength fluorescence imaging in living mammalian cells: effects of mitotic inhibitors on cell cycle progression. *Genes Cells* 1997; **2**(6): 369-80.

[20] Hima CR, Manimegalai M. Studies on larvicidal activity of *Gloriosa superba* on the developmental stages of the chikungunya mosquito, *Aedes aegypti*. *Int J Curr Res* 2014; **6**(5): 6687-90.