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EVALUATION OF *DRACAENA LOUREIRI* STEM AND LEAF EXTRACTS FOR THEIR LARVICIDAL ACTIVITY AGAINST *AEDES AEGYPTI*

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ABSTRACT

Insecticides have long been utilized to combat mosquito-borne diseases, but concerns over environmental impact and resistance have prompted exploration of plant-derived alternatives. This study assessed the larvicidal potential of stem and leaf extracts obtained from *Dracaena loureiri.* Crude ethanolic extracts underwent larvicidal activity testing against *Aedes aegypti* larvae. The crude leaf extract exhibited significant efficacy, with LC_{50} values of 309.71 mg/ l (24 h) and 232.58 mg/ l (48 h), whereas the stem extract displayed no activity. Fractionation by column chromatography was performed on the crude extract exhibiting promising activity, followed by a re-evaluation of its efficacy. Two fractions obtained from the leaf extract demonstrated potent larvicidal properties: RC-DT 038 yielded LC_{50} values of 163.15 mg/ l (24 h) and 138.01 mg/ l (48 h), while RC-DT 040 exhibited LC_{50} values of 274.25 mg/ l (24 h) and 257.65 mg/ l (48 h). The results suggest the presence of larvicidal bioactive compounds specific to the leaves.

Key words: *Dracaena loureiri*, phytochemical, plant extract, ethanolic extract, crude extract, column chromatographic fractionation, mosquito control, insecticide, *Aedes aegypti*, larvicidal activity

Mosquito-borne diseases continue to pose significant public health challenges, requiring effective vector control strategies. Traditional insecticides, such as temephos, have been widely used due to their efficacy in reducing mosquito larvae populations. However, the prolonged use of temephos has led to negative impacts on non-target organisms and the emergence of resistance in mosquito populations (Chareonviriyaphap et al., 2013; Gan et al., 2021; Zulfa et al., 2022; Sumitha et al., 2023). These concerns underscore the urgent need for alternative, environmentally friendly larvicides that can reduce these issues. Plant-derived substances have emerged as promising alternatives to synthetic insecticides, because of their low toxicity, easy biodegradability, and environmental safety (Benelli, 2016; Piplani et al., 2019; Chaudhari et al., 2021). Specifically, several studies have demonstrated the larvicidal potential of plant extracts against *Aedes aegypti*, the primary vector of dengue, Zika, and chikungunya viruses (Luz et al., 2020; Silverio et al., 2020; Marin et al., 2023). In this context, the garden tree *Dracaena loureiri* Gagnep, commonly grown in Thailand, causes attention for its potential application in mosquito control. While previous research has demonstrated the insecticidal properties of *D. loureiri* endocarp extract against various mosquito species, including *Ae. aegypti* (Thongwat et al., 2017; 2018), there is a lack of studies exploring the larvicidal activity of other plant parts, particularly the stem and leaves. Given that *D. loureiri* is a fast-growing plant that produces abundant stems and leaves throughout the year, these parts could serve as a readily available and sustainable source of bio-larvicides. Therefore, this study aims to evaluate the larvicidal activity of stem and leaf extracts of *D. loureiri* against *Ae. aegypti* mosquito. If effective, these extracts could be developed into environmentally friendly bio-larvicides, providing a valuable tool for sustainable mosquito control.

MATERIALS AND METHODS

Fresh samples of natural growing *D. loureiri* stems (5 kg) and leaves (1.6 kg) were obtained from Phitsanulok Province, Thailand. Voucher specimens DTNU012 (stems) and DTNU013 (leaves) were deposited at the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Thailand. The preparation and extraction were followed methods of Thongwat et al. (2018).The samples were washed with tap water, air dried and placed in a hot air

oven (260M Lab Oven, Contherm, Lower Hutt, New Zealand) at 45°C for 3 days. The dried stems (891.75 g) and leaves (343.45 g) were ground to powder in an electric blender at 22,000 rpm (800G instrument; MRC Laboratory-Instruments, Essex, UK), and then macerated with absolute ethanol (1 g:100 ml) in a rotary shaker (Platform Shaker, New Brunswick™Innova® 2300, Eppendorf, Hamburg, Germany) at 180 rpm for 24 hours. The suspension was subjected to filtration using a Buchner funnel and Whatman™ No. 1 filter paper. Subsequently, the filtrates were subjected to dryness in rotary vacuum evaporator (Hei-vap value, Heidolph Instruments GmbH and Co. KG, Schwabach, Germany) and dried completely in the hot air oven maintained at 45°C. The resulting dry extract was stored in a desiccator. After that, the ethanol extract (11.0 g) was fractionated by column chromatography using Merck silica gel 60 (0.063–0.200 mm, 250 g) with a gradient solvent system ranging from hexane to ethyl acetate. The proportion of the more polar solvent (ethyl acetate) was gradually increased by 5% v/v at each step. The eluates were then examined by Thin Layer Chromatography (TLC) using Silica gel 60 F_{254} (Merck), resulting in the acquisition of eight combined fractions (RC-DT 036 - RC-DT 043), which were subsequently stored under 4°C conditions.

Aedes aegypti mosquitoes were sourced from the laboratory strain of the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Thailand. The mosquitoes were reared in controlled conditions [25±2 °C, 70-80% relative humidity, and 10:14 (light: dark) photo-period]. They were raised as larvae in a white plastic tray filled with tap water and fed powdery dog biscuits (Adult Complete Nutrition, Pedigree®, Mars Petcare, Franklin, TN). The pupae were kept in a mosquito cage (30 x 30 x 30 cm) until they developed into adults. Once emerged, the adult mosquitoes were given a solution of 5% sugar and 5% multivitamin syrup (Seven Seas®, Feltham, UK) to feed on. After 5 days, the female mosquitoes were provided with an artificial membrane to take a blood meal (Rutledge et al., 1964). Once they became gravid, they were allowed to lay eggs on a filter paper. The eggs were then air-dried and stored in a humidity-controlled glass jar until they were required. The eggs were hatched and the larvae were reared under the conditions described until they were ready for the bioassay.

The bioassay was conducted on 3rd instar *Ae. aegypti* larvae, following the standard protocols outlined by the World Health Organization (WHO, 2005). A stock solution of each sample was prepared at a concentration of 2% w/v in dimethyl sulfoxide (DMSO) and stored at 4°C until required. The stock solution was serially diluted in 100 ml of tap water, resulting in concentrations of 200-500 mg/ l for the crude extracts (both stems and leaves). Preliminary testing of the eight fractions was performed at a concentration of 200 mg/ l, followed by testing of selected fractions at concentrations ranging from 50 to 450 mg/ l. These solutions were prepared in food-grade plastic bowls with dimensions of 7 cm dia x 4.5 cm height. Third instar larvae (n = 25) were exposed to the assay solutions, and mortality rates were determined at 24- and 48-hr post-exposure, during which no food was provided to the larvae. A larva was deemed dead when it was unable to move normally after being lightly touched with a brush. Each diluted sample was subjected to three independent experiments, with each experiment carried out in quadruplicate. A control was prepared in tap water using only DMSO, with highest concentration matching that of the tests. The larvicidal data were analyzed using probit analysis for the determination of 50 % (LC_{50}) lethal value, in accordance with the method described by Finney (1971). The analysis was performed using the Ldp-line software (Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt), with fiducial confidence intervals of 95% reported, comprising the lower confidence limit (LCL) and upper confidence limit (UCL). The LC_{50} values were considered significantly different when the ranges between LCL and UCL did not overlap.

RESULTS AND DISCUSSION

The yield of the crude ethanol extract of *D. loureiri* stems and leaves was 1.30% (w/w) (65.09 g from 5.0 kg) and 1.62% (w/w) (26.05 g from 1.6 kg), respectively. After bioassays, silica gel 60 column chromatography of the crude leaves extract (22.0 g) generated 150 eluted fractions, which were pooled into six fractions, namely, RC-DT 036 (fractions 1-34) containing 0.09 g of extract, RC-DT 037 (fractions 35-50) 1.44 g, RC-DT 038 (fractions 51-66) 0.94 g, RC-DT 039 (fractions 67-86) 1.73 g, RC-DT 040 (fractions 87-96) 0.86 g, RC-DT 041 (fractions 97-111) 1.87 g, RC-DT 042 (fractions 112-141) 2.56 g, and RC-DT 043 (fractions 142-150) 3.47 g. Upon comparison of the crude ethanol extracts obtained from *D. loureiri*, marked differences in efficacy were observed. Specifically, the extract derived from the leaves of the plant exhibited significant larvicidal activity against 3rd instar larvae of *Ae. aegypti*, with LC_{50} values of 309.71 mg/ l (24 h) and 232.58 mg/

l (48 h). In contrast, the extract obtained from the stems of the plant did not exhibit any significant larvicidal activity, resulting in zero observed mortality. Then, third instar *Ae. aegypti* larvae were exposed to leaf fraction extracts at a concentration of 200 mg/l for a period of 24 hours. The larval mortality rates observed for each fraction were 0, 0, 60, 17, 39, 18, 1, and 0%, for RC-DT 036 to RC-DT 043, respectively. Further investigation was focused on RC-DT 038 and RC-DT 040 due to their distinct mortality rates and polarity characteristics. RC-DT 038, with a 60% mortality rate, is moderately polar, which may influence its effectiveness against larvae. RC-DT 040, with a 39% mortality rate and a moderately to highly polar composition, also showed significant effects. The polarity of these fractions suggests that they contain active compounds that are more soluble in polar environments, which could be crucial for their larvicidal activity. These characteristics, combined with their mortality rates, make them promising candidates for further analysis.

Table 1 presents the results of the larvicidal efficacy of RC-DT fractions 038 and 040. When compared to the crude leaves extract of *D. loureiri*, it showed that RC-DT 038 has significantly higher larvicidal activity than the crude leaves extract, with the LC_{50} values of 163.15 mg/ l and 138.01 mg/ l for 24- and 48-hr exposure times, respectively. This suggests that RC-DT 038 contains compounds with strong larvicidal properties against *Ae. aegypti* larvae. On the other hand, the LC_{50} values

Table 1. Larvicidal activity of *Dracaena loureiri* extracts against the 3rd instar larvae of *Aedes aegypti*

Fraction	Concentration (mg/ l)	24-hour exposure time		48-hour exposure time	
		% mortality (± SD)	LC_{50} (LCL-UCL) (mg/ l)	% mortality (± SD)	LC_{50} (LCL-UCL) (mg/ l)
Leaves crude extract[a]	Control	0	309.71 (300.29-318.95)	2.00± 2.31	232.58 (190.67-253.02)
	200	19.44± 4.92		40.47± 12.82	
	250	33.65± 10.98		54.59± 13.03	
	300	47.49± 8.08		65.78± 9.85	
	350	59.53± 10.98		74.31± 10.65	
	400	69.29± 9.20		80.68± 11.11	
	450	76.92± 4.33		85.41± 5.69	
	500	82.73± 5.91		88.93± 5.24	
RC-DT038	Control	0	163.15 (148.32-177.77)	0	138.01 (125.07-150.60)
	50	9.79± 5.03		11.33± 5.16	
	100	29.66± 2.31		35.06± 3.27	
	150	46.42± 6.83		53.95± 2.31	
	200	58.92± 3.27		67.07± 4.00	
	250	68.08± 5.03		76.04± 5.03	
	300	74.85± 6.83		82.24± 5.66	
	350	79.92± 5.03		86.61± 5.03	
	400	83.77± 3.83		89.75± 6.00	
	450	86.73± 3.27		100	
RC-DT040	Control	0	274.25 (230.67-333.94)	0	257.65 (195.94-353.85)
	50	2.92± 2.00		4.12± 3.83	
	100	10.84± 6.00		15.79± 3.83	
	150	23.00± 10.83		28.32± 5.74	
	200	34.95± 8.64		39.41± 8.87	
	250	45.48± 10.52		48.72± 9.80	
	300	54.37± 8.87		56.40± 8.87	
	350	61.74± 12.44		62.73± 8.25	
	400	67.81± 6.00		67.95± 3.83	
	450	72.79± 3.83		72.28± 3.27	

[a]For 48-hr exposure time, because mortality of the control was found, corrected mortalities were used; Significantly different LC_{50} values (p<0.05) can be determined from the LCL-UCL. Between two extracts, if the LCL-UCL values of each extract overlap, there is no significant difference. If not, there is significant difference; C_{50}: 50% lethal concentration; LCL: lower confidence limits; mg/l: milligram per liter; SD: standard deviation; UCL upper confidence limits

of RC-DT 040 were also higher than those of the crude leaves extract, but the difference was not statistically significant. The LC_{50} values for RC-DT 040 were 274.25 mg/ l (24 h) and 257.65 mg/ l (48 h), indicating that this fraction may contain compounds with moderate larvicidal properties.These suggest that the bioactive constituents responsible for the observed larvicidal properties are likely concentrated solely within the leaves and fruit endocarps of the plant (Thongwat et al., 2017; 2018). In comparison to the previous study by Thongwat et al. (2018) the leaf extract in this study exhibited slightly lower activity than the fruit endocarp extract, with LC_{50} values of 309.17 and 224.73 mg/ l, respectively. This observation leads to the inference that both the fruit endocarp and leaf of *D. loureiri* may contain similar compounds that effectively target mosquito larvae. Additionally, comparison with other studies that focus on the stem wood extract of the plant revealed that the chemical composition of *D. loureiri* extract primarily comprises phenolic compounds and flavonoids, including loureirins, loureiriol, and stilbenoid derivatives (Meksuriyenand Cordell, 1988; Likhitwitayawuid et al., 2002; El-Halawany et al., 2011; Thu et al., 2020; Huang et al., 2024). Consequently, it may be deduced that the chemical constituents responsible for mosquito larvicidal activity are not those chemicals reported from the stem wood. Further investigation into the chemical composition of the *D. loureiri* leaf and fruit endocarps is warranted to elucidate the active components contributing to its observed insecticidal properties. Such exploration may lead to the discovery of novel mosquito control agents.

The presence or absence of active compounds in different parts of a plant can be attributed to several factors, including genetic makeup, developmental stage, and environmental conditions. For example, it is possible that the genes responsible for the production of the active compounds are only expressed in the leaves but not in the stems (Li et al., 2020). Additionally, the environmental conditions such as sunlight exposure, temperature, and humidity can also affect the production and accumulation of active compounds in different plant parts (Anupam et al., 2012; Kumar et al., 2023). The lack of activity observed in the stem extract highlights the importance of selecting the correct plant part for extraction in order to obtain the desired bioactivity. Understanding the factors influencing the distribution of bioactive compounds within a plant is crucial for optimizing the extraction of valuable compounds for various applications, including pest control. The year-round availability of *D. loureiri* leaves provides a consistent and accessible resource for larvicidal applications against mosquitoes. This accessibility ensures a reliable supply of raw material for sustainable mosquito control efforts, reducing dependence on seasonal variations or limited availability. Leveraging locally abundant *D. loureiri* leaves supports community-based vector control initiatives, enhancing community resilience and empowerment. Collaboration between researchers, policymakers, and communities is essential to harness the full potential of *D. loureiri* leaves for effective and sustainable mosquito control.

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AUTHOR CONTRIBUTION STATEMENT

Damrongpan Thongwat: Study design, Literature search, Experimental studies, Data analysis, Manuscript preparation and editing. Lucksagoon Ganranoo and Ratchanaporn Chokchaisiri: Study design, Experimental studies, Data analysis, Manuscript editing. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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