TROPICAL LIFE SCIENCES RESEARCH

Efficiency of Dried *Cassia fistula* L. Pod Extracts against Dengue Vector, *Aedes aegypti* L. (Diptera: Culicidae) via Larvicidal, Pupicidal and Adulticidal Potentials

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Highlights

- Danthron (1,8-dihydroxy-3-anthraquinone) was detected in the fruit peel of *Cassia fistula* for the first time.

- Methanolic extract of *C. fistula* fruit peel showed strong efficacy against *Aedes aegypti*.

- *C. fistula* fruit peel extract may serve as a low-cost, eco-friendly alternative to synthetic mosquito control agents.

EARLY VIEW

Efficiency of Dried *Cassia fistula* L. Pod Extracts against Dengue Vector, *Aedes aegypti* L. (Diptera: Culicidae) via Larvicidal, Pupicidal and Adulticidal Potentials

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Abstract: The growing resistance of *Aedes* mosquitoes to synthetic insecticides and the environmental hazards associated with traditional management approaches underscore the pressing necessity for biodegradable alternatives. This research assessed the bioefficacy of *Cassia fistula* pod extracts (seed, fruit pulp, and fruit peel) against *Aedes aegypti* at various developmental stages (larvae, pupae, and adults) and revealed significant anthraquinone chemicals. Powdered pod materials were extracted using 70% methanol and ethyl acetate using Soxhlet extraction, and their chemical components were analyzed using LC-MS. Bioassays were performed on both laboratory and field strains of *A. aegypti* was tested with 25 larvae and 25 pupae at each concentration (four replicates), and adult mortality was measured after 24 hours using WHO-modified tube tests. We performed probit analysis to find the LC_{50} and LC_{90} values. LC-MS profiling identified seven anthraquinones: physcion-8-O-β-D-glucoside, sennoside, aloin, chrysophanol-8-O-D-glucoside, aloe-emodin, emodin-8-O-β-D-glucoside, and notably, danthron, which was exclusively found in the fruit peel and pulp. The methanolic fruit peel extract had the highest larvicidal and pupicidal activity of all the extracts, with 24-hour LC_{50} values of 92.80 mg/L and 102.12 mg/L, respectively. Moderate adulticidal efficacy (44.00% at 200 mg/L) was noted, with efficacy ranking as follows: fruit peel > fruit pulp > seed. The reduced effectiveness against adult mosquitoes may be due to their thicker, more sclerotized cuticle and elevated detoxification enzyme expression. These results indicate that *C. fistula*, especially its fruit peel, shows potential as a powerful, eco-friendly larvicide and pupicide, making it a sustainable plant-based option for use in programs to control dengue vectors.

Keywords: Anthraquinone, *Cassia fistula* Pod, Larvicide, Pupicide, Adulticide, *Aedes aegypti*

INTRODUCTION

Aedes mosquitoes serve as the primary vectors of significant arboviruses, including dengue hemorrhagic fever, chikungunya fever, yellow fever, and Zika virus infection. The World Health Organization indicated that in 2024, there were over 7.6 million cases of dengue globally. The data revealed 3.4 million confirmed cases, over 16,000 severe cases, and more than of 3,000 fatalities. While vaccines for dengue are available, the most effective strategies for protection continue to focus on vector control and preventing bites (WHO, 2024). Nevertheless, the widespread application of synthetic pyrethroids and organophosphates has led to the development of insect resistance, environmental pollution, and potential risks to human health.

Consequently, an increasing number of people are seeking sustainable and cost-effective alternatives. Compounds derived from plants, particularly those abundant in bioactive phytochemicals, are gaining importance due to their potential to eliminate insects and larvae, as well as to repel them (Jayaraman, Senthilkumar, & Venkatesalu, 2015; Ramkumar, Karthi, Muthusamy, Natarajan, & Shivakumar, 2015; Tong & Bloomquist, 2013). Anthraquinones, especially 9,10-anthraquinone (AQ), represent a group of functionally diverse aromatic compounds that can be found across various plant families, fungi, and lichens (Dave & Ledwani, 2012; Soto-Blanco, 2022). These applications are presently utilized in pharmaceuticals, cosmetics, food, and pest control (Dave & Ledwani, 2012).

Cassia fistula Linn. The golden shower tree (Fabaceae) is Thailand's national tree and flower. It is native to South Asia and is frequently utilized in traditional medicine to treat malaria and fever. The plant has anthraquinones, flavonoids, polysaccharides, and mucilage it contains (Dave & Ledwani, 2012). Previous studies have demonstrated the exclusive larvicidal efficacy of *Cassia* fruit and leaf extracts against *Culex pipiens* (Abutaha, Al-Mekhlafi, & Farooq, 2020; Fouad et al., 2018). *C. quinquefasciatus* (Ullah, Ijaz, Mughal, & Zia, 2018), *Ae. albopictus* (Fouad et al., 2018) and *Anopheles stephensi* (Rajkumar & Jebanesan, 2009). Nevertheless, there is insufficient data about the larvicidal, pupicidal, and adulticidal effects of *C. fistula* fruit pulp and peel, especially against *Ae. aegypti*. The objectives of this study were to evaluate the bioefficacy of extracts from *C. fistula* pods (fruit pulp, peel, and seed) against *Ae. aegypti* at various developmental stages (larva, pupa, and adult), and to describe their anthraquinone content derived from *C. fistula* pods. The findings of this study might help in the development of plant-derived alternatives for mosquito control.

MATERIAL AND METHOD

Aedes Mosquitoes Rearing

Two strains of *Ae. aegypti* were used in this study: a laboratory strain (*Ae. aegypti* strain Bora Bora) and a field strain. The laboratory strain originated from the insectarium of the Office of Disease Prevention and Control 7, Khon Kaen, Thailand, where it has been cultivated since the 1990s. The field strain larvae obtained from man-made containers around Mahasarakham University, Thailand (16°11′57″N 103°16′59″E). Applying identical techniques as the first group, reared in the lab. Pictorial keys for the identification of *Aedes* mosquito larvae and adults based on externally morphological characteristics according to Rueda (2004). Eggs were collected on filter paper and submerged in 1 L of seasoned water to stimulate hatching. Larvae were reared under controlled insectary conditions (27 ± 2 °C, 70 ± 10% relative humidity, and a 12:12 h light-dark cycle) and fed with rodent food (Smart Hearth, Thailand) at 1 g/daily. Pupae were transferred to dechlorinated water in screened cages (30 × 30 × 30 cm) for adult emergence (F_0 generation). Adults (F_0) were maintained on cotton pads soaked in Multi-Vitamin Syrup (Seven Seas, Thailand), and females were blood-fed using guinea pigs three times per week. Two days after blood feeding, oviposition was encouraged using cone-shaped Whatman No.1 filter papers moistened with 5 mL of water. The resulting eggs were used to establish the F_1 generation, which was subsequently used for bioassay experiments.

Preparation of Plant Extract

Mature fruits (pods) of *C. fistula* were randomly collected from trees in Kantarawichai District, Mahasarakham Province, Thailand (16°19′22″N, 103°17′48″E). They were identified by botanist at the Department of Biology, Faculty of Science, Mahasarakham University and identified according to Patole (2013). There were three parts to the *C. fistula* pods: seeds, fruit pulp, and peels. All the parts were dried in the shade for 30 days and then in the oven at 100 °C for 48 hours. We used a stainless-steel blender to grind the dried materials and then ran them through a 2 mm mesh screen. The powdered material was sealed in polyethylene bags and stored at 0 °C until use. For extraction, 50 g of each plant part was separately processed using Soxhlet extraction with 70% ethyl acetate and 70% methanol (2,000 mL each). The process was repeated three times until the solvent became nearly colorless. Crude extracts were concentrated using a rotary vacuum evaporator and stored at 4 °C for further analysis.

Examination of Anthraquinone Substance

Anthraquinone (Sigma, USA) was used as the standard for quantitative analysis. The calibration curve of standard was made from 5 concentrations (3 replications). All concentrations of both plant species were measured by Liquid Chromatography-Mass Spectrometer (Thermo MSQ Plus LC-MS with Accela HPLC).

Larvicidal Bioassays

The larvicidal activity was evaluated in accordance with WHO criteria (WHO, 2005). Late third- and early fourth-instar larvae of *Ae. aegypti* (both laboratory and field strains) were utilized. Preliminary tests were conducted to determine appropriate concentration ranges of *C. fistula* extracts (seed, pulp, and peel). Temephos (1 mg/L) was the positive control, and distilled water (100 mL) was the negative control. There were four replicates of each treatment, and 25 larvae had been exposed to concentration of 50, 75, 100, 150, and 200 mg/L. After 24 hours of exposure, the number of deaths was reported.

Pupacidal Bioassays

The pupicidal activity of *C. fistula* extracts (seed, pulp, and peel) was evaluated against newly emerged *Ae. aegypti* pupae. Twenty-five pupae were exposed to each concentration (50, 75, 100, 150, and 200 mg/L) in four replicates. Controls were prepared as described in the larvicidal assay. After 24 hours of exposure, mortality was recorded and expressed as a percentage.

Adulticidal Bioassays

Five-day-old female *Ae. aegypti* mosquitoes, previously fed with Multi-Vitamin Syrup, were utilized in adulticidal assays according to a modified WHO procedure (WHO, 2016). The apparatus used for testing was made up of two plastic tubes that were 125 mm long and 44 mm wide. *C. fistula* extracts (seed, pulp, and peel) were diluted in dimethyl sulfoxide (DMSO) to final concentrations of 100, 150, and 200 mg/L. Filter papers (140 × 120 mm) were saturated with the diluted extracts. Deltamethrin-impregnated paper (0.05%) served as the positive control, and DMSO-saturated paper was used as the negative control.

Mosquitoes were introduced into the exposure tube containing treated paper, while control groups were placed in identical tubes. Each treatment consisted of four replicates with 25 female mosquitoes per replicate. Assessing the mortality at 24 hours, while the dead mosquitoes were brought out. After 24 hours of exposure, mosquitoes were transferred to holding tubes and maintained with cotton pads soaked in Multi-Vitamin Syrup. Mortality was assessed after 24 hours. If a mosquito could no longer stand, it was considered dead.

Statistical Analysis

Institution Animal Care and Use Committee (IACUC) of Mahasarakham University, Maha Sarakham, Thailand, approved the use of animals under this study (IACUC-MSU-44/2023). Larval, pupal, and adult mortality data were analyzed using probit regression (SPSS v.24, Chicago, IL, USA) to calculate LC_{50} and LC_{90} values. Mean mortality rates were compared using one-way ANOVA followed by LSD post hoc tests. Differences were considered statistically significant at $p < 0.05$. Moreover, insecticide susceptibility was classified based on the criteria that 98%–100% mortality of mosquitoes implies susceptibility, 80%-97% mortality indicates possible resistance that needs to be further confirmed via biochemical or molecular assays, and less than 80% mortality implies resistance (WHO, 2016).

RESULTS

By applying ethyl acetate and methanol solvents, the yield (%) of plant extracts were calculated and the results are presented in Table 1. The methanolic extract of *C. fistula* fruit pulp showed the largest quantity of plant material (91.88%), whereas the lowest yield was obtained in the ethyl acetate extract of *C. fistula* fruit peel (12.52%). The worthiness of extracting compound of fruit pulp methanolic extract was considered.

Table 1: The plant extract yield (%) in ethyl acetate and methanol extracts of *C.fistula* (seed, fruit pulp, and fruit peel).

C. fistula	Mass of the extract yield (%)	
	Ethyl acetate	Methanol
Seed	22.10	36.18
Fruit Pulp	18.30	91.88
Fruit Peel	12.52	35.20

Phytochemical Analysis

LC-MS profiling revealed the presence of seven major anthraquinones across the different extracts (Table 2). These included physcion-8-O-β-D-glucoside, sennoside, aloin, chrysophanol-8-O-D-glucoside, aloe-emodin, emodin-8-O-β-D-glucoside, and 1,8-dihydroxy-3-anthraquinone (Danthron). Chrysophanol-8-O-D-glucoside was detected only in the seed and peel extracts, while Danthron was uniquely present in the pulp and peel extracts.

Table 2*:* Retention time (RT) and major anthraquinone compounds identified in *C. fistula* extracts via LC-MS.

Peak no.	RT (min)	Compound name	$[M+H]^+$	Detected in
1	5.95	Physcion-8-o-β-D-glucoside	445	seed, fruit pulp, peel
2	6.15	Sennoside	861	seed, fruit pulp, peel
3	8.42	Aloin	417.09	seed, fruit pulp, peel
4	8.77	Chrysophanol-8-o- D-glucoside	415	seed, peel
5	12.65	Aloe-emodin	269.20	seed, fruit pulp, peel
6	13.59	1,8-dihydroxy-3-anthraquinone (Danthron)	241.09	fruit pulp, peel
7	15.28	Emodin-8-o- β-D-glucoside	431	seed, fruit pulp, peel

Aedes Larvicidal Activity

The larvicidal efficacy of *C. fistula* pod extracts (seed, fruit pulp, and fruit peel) was evaluated against third- to fourth-instar *Ae. aegypti* larvae from both laboratory and field strains at concentrations ranging from 50 to 200 mg/L. Mortality was recorded after 24 hours, and LC_{50} and LC_{90} values were calculated using probit regression analysis (Table 3-4). Negative controls (distilled water) resulted in 0% mortality, while positive controls (1 mg/L temephos) induced 100% mortality in both strains, confirming assay validity. Among the six tested extracts, the methanolic extract of fruit peel exhibited the most potent larvicidal activity, achieving 100.00 ± 0.00% mortality in laboratory strain larvae and 90.00 ± 5.16% in field strain larvae at 200 mg/L. This was followed by the methanolic extract of fruit pulp and the ethyl acetate extract of fruit peel.

Table 3: Larvicidal activity (% mortality ± SD) of *C. fistula* extracts against *Ae. aegypti* (laboratory strain) after 24 h exposure.

Plant samples	control	50 mgL^{-1}	75 mgL^{-1}	100 mgL^{-1}	150 mgL^{-1}	200 mgL^{-1}	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)
Seed / Ethyl acetate	0.00 ± 0.00	8.00 ± 3.27 a	19.00 ± 2.00 a	33.00 ± 6.83 a	65.00 ± 6.00 a	78.00 ± 5.16 a	124.36 (115.32 - 134.99)	270.13 (233.37 - 329.56)
Seed / Methanol	0.00 ± 0.00	9.00 ± 2.00 a	20.00 ± 3.27 a	41.00 ± 8.87 a	72.00 ± 3.27 ab	89.00 ± 6.83 b	110.18 (103.06 - 117.99)	215.29 (192.35 – 249.31)
Fruit Pulp / Ethyl acetate	0.00 ± 0.00	9.00 ± 3.83 a	22.00 ± 4.00 b	45.00 ± 2.00 b	74.00 ± 5.16 b	89.00 ± 3.83 b	107.03 (100.08 – 114.57)	209.75 (187.66 - 242.41)
Fruit Pulp / Methanol	0.00 ± 0.00	10.00 ± 5.16 a	25.00 ± 3.83 bc	47.00 ± 5.03 b	78.00 ± 7.66 b	93.00 ± 2.00 b	101.34 (94.95 – 108.14)	191.96 (173.28 – 218.96)
Fruit Peel / Ethyl acetate	0.00 ± 0.00	12.00 ± 3.27 ab	25.00 ± 2.00 bc	48.00 ± 3.27 b	77.00 ± 5.03 b	92.00 ± 4.62 b	100.88 (94.25 - 107.94)	198.41 (177.91 - 228.56)
Fruit Peel / Methanol	0.00 ± 0.00	16.00 ± 3.27 b	28.00 ± 3.27 c	52.00 ± 5.66 b	79.00 ± 6.83 b	100.00 ± 0.00 c	92.80 (71.22 - 118.18)	173.83 (132.34 - 341.82)
Temephos 1 mgL^{-1} 100.00 ± 0.00								

Note: Mean of 4 replications followed by the different lower-case letters within the same column (treatment concentration only) represent statistically significantly differences at $p < 0.05$ using one-way ANOVA and followed by LSD test. LC_{50}, lethal concentration revealing 50% mortality; LC_{90}, lethal concentration revealing 90% mortality; LCL, lower confidence limits; and UCL, upper confidence limits.

Table 4: Larvicidal activity (% mortality ± SD) *of C. fistula* extracts against *Ae. aegypti* (field strain) after 24 h exposure.

	control	50 mgL^{-1}	75 mgL^{-1}	100 mgL^{-1}	150 mgL^{-1}	200 mgL^{-1}	LC_{50} (95%CI)	LC_{90} (95%CI)
Seed / Ethyl acetate	0.00 ± 0.00	4.00 ± 3.27 a	13.00 ± 2.00 a	26.00 ± 4.00 a	55.00 ± 3.83 a	61.00 ± 3.83 a	153.29 (140.24 – 170.81)	356.82 (294.41 – 470.15)
Seed / Methanol	0.00 ± 0.00	5.00 ± 2.00 ab	13.00 ± 3.83 a	31.00 ± 6.83 a	56.00 ± 3.27 a	69.00 ± 3.83 b	141.55 (130.55 – 155.44)	314.57 (266.07 – 397.43)
Fruit Pulp / Ethyl acetate	0.00 ± 0.00	5.00 ± 2.00 ab	14.00 ± 5.16 a	39.00 ± 3.83 b	59.00 ± 5.03 a	72.00 ± 5.66 b	132.55 (122.68 – 144.54)	290.78 (248.86 – 360.38)
Fruit Pulp / Methanol	0.00 ± 0.00	8.00 ± 3.27 ab	22.00 ± 2.31 bc	40.00 ± 3.27 b	69.00 ± 6.83 b	79.00 ± 3.83 c	117.14 (108.69 – 126.76)	254.60 (221.24 – 307.81)
Fruit Peel / Ethyl acetate	0.00 ± 0.00	9.00 ± 2.00 b	19.00 ± 2.00 b	41.00 ± 3.83 b	69.00 ± 6.00 b	83.00 ± 3.83 c	115.18 (107.21 – 124.14)	240.24 (211.06 – 285.46)
Fruit Peel / Methanol	0.00 ± 0.00	14.00 ± 5.16 c	25.00 ± 2.00 c	50.00 ± 2.31 c	76.00 ± 5.66 b	90.00 ± 5.16 d	100.47 (93.41 – 107.90)	207.70 (184.51 – 242.68)
Temephos 1 mgL^{-1} 100.00 ± 0.00								

Note: Mean of 4 replications followed by the different lower-case letters within the same column (treatment concentration only) represent statistically significantly differences at $p < 0.05$ using one-way ANOVA and followed by LSD test. LC_{50}, lethal concentration revealing 50% mortality; LC_{90}, lethal concentration revealing 90% mortality; LCL, lower confidence limits; and UCL, upper confidence limits.

Statistical analysis revealed significant differences between treatments. The methanolic fruit peel extract was the most effective when it was considered had the lowest LC_{50} and LC_{90} values: 92.80 and 173.83 mg/L for the laboratory strain and 100.47 and 207.70 mg/L for the field strain (Table 7). Across all samples, the rank order of larvicidal effectiveness was consistent: **fruit peel > fruit pulp > seed**. Additionally, laboratory strain larvae were more susceptible than field strain counterparts, reflecting potential resistance factors in wild populations.

Pupicidal Activity

The negative control group had no pupa deaths, whereas the positive control group (temephos 1 mg/L) had 100% deaths, which confirmed that the assay was accurate. Among all extracts, the methanolic fruit peel extract demonstrated the strongest pupicidal effect, causing 88.00 ± 3.27% and 80.00 ± 3.27% mortality in laboratory and field strain pupae, respectively, at 200 mg/L. This was followed by fruit pulp extracts, with seed extracts consistently showing the weakest effect. The methanolic fruit peel extract also exhibited the lowest LC_{50} and LC_{90} values were 102.12 and 213.89 mg/L (lab strain), and 111.59 and 244.89 mg/L (field strain) (Table 5-6). Statistical analysis confirmed significant differences across treatments. Pupae from the laboratory strain were more susceptible than the field strain, mirroring trends in the larval assays. The overall efficacy followed the consistent trend: fruit peel > fruit pulp > seed, indicating that the methanolic fruit peel extract contains the most potent pupicidal constituents and holds promise as a botanical alternative for mosquito control (Table 7).

Table 5: Pupicidal activity of different solvent extracts of *C. fistula* against *Ae. aegypti* (laboratory strain).

	control	50 mgL^{-1}	75 mgL^{-1}	100 mgL^{-1}	150 mgL^{-1}	200 mgL^{-1}	LC_{50} (95%CI)	LC_{90} (95%CI)
Seed / Ethyl acetate	0.00 ± 0.00	3.00 ± 2.00 a	11.00 ± 3.83 a	24.00 ± 3.27 a	46.00 ± 2.31 a	62.00 ± 5.16 a	161.61 (147.64 – 180.81)	366.57 (301.97 – 484.82)
Seed / Methanol	0.00 ± 0.00	4.00 ± 3.27 ab	10.00 ± 2.31 a	27.00 ± 3.83 a	52.00 ± 7.30 a	68.00 ± 3.27 ab	148.63 (137.19 – 163.31)	318.78 (270.44 – 401.05)
Fruit Pulp / Ethyl acetate	0.00 ± 0.00	6.00 ± 2.31 ab	16.00 ± 3.27 b	39.00 ± 6.83 b	61.00 ± 3.83 b	71.00 ± 5.03 b	130.99 (120.93 – 143.23)	297.43 (252.61 – 373.15)
Fruit Pulp / Methanol	0.00 ± 0.00	9.00 ± 3.83 bc	21.00 ± 2.00 c	45.00 ± 3.83 bc	61.00 ± 6.00 b	74.00 ± 8.33 bc	122.64 (112.75 – 134.46)	298.64 (250.77 – 381.86)
Fruit Peel /	0.00 ± 0.00	9.00 ± 2.00 bc	22.00 ± 2.31 c	45.00 ± 2.00 c	70.00 ± 5.16 c	80.00 ± 3.27 c	113.46 (105.24 – 122.68)	247.96 (215.83 – 298.96)

Ethyl acetate								
Fruit Peel / Methanol	0.00 ± 0.00	14.00 ± 5.16 c	23.00 ± 3.83 c	50.00 ± 5.16 c	76.00 ± 3.27 c	88.00 ± 3.27 d	102.12 (94.95 – 109.80)	213.89 (189.36 – 251.20)
Temephos 1 mgL^{-1} 100.00 ± 0.00								

Note: Mean of 4 replications followed by the different lower-case letters within the same column (treatment concentration only) represent statistically significantly differences at $p < 0.05$ using one-way ANOVA and followed by LSD test. LC_{50}, lethal concentration revealing 50% mortality; LC_{90}, lethal concentration revealing 90% mortality; LCL, lower confidence limits; and UCL, upper confidence limits.

Table 6: Pupicidal activity of different solvent extracts of *C. fistula* against *Ae. aegypti* (field strain).

	control	50 mgL^{-1}	75 mgL^{-1}	100 mgL^{-1}	150 mgL^{-1}	200 mgL^{-1}	LC_{50} (95%CI)	LC_{90} (95%CI)
Seed / Ethyl acetate	0.00 ± 0.00	3.00 ± 2.00 a	9.00 ± 2.00 a	18.00 ± 2.31 a	33.00 ± 3.83 a	57.00 ± 3.83 a	185.87 (167.31 – 213.93)	429.01 (342.09 – 602.32)
Seed / Methanol	0.00 ± 0.00	3.00 ± 2.00 a	9.00 ± 2.00 a	22.00 ± 5.16 a	38.00 ± 5.16 a	58.00 ± 5.16 a	177.19 (159.93 – 202.67)	418.59 (334.78 – 583.45)
Fruit Pulp / Ethyl acetate	0.00 ± 0.00	4.00 ± 3.27 a	10.00 ± 2.31 a	30.00 ± 4.00 b	53.00 ± 3.83 b	60.00 ± 8.64 a	154.93 (141.62 – 172.91)	361.44 (297.50 – 478.36)
Fruit Pulp / Methanol	0.00 ± 0.00	5.00 ± 2.00 ab	17.00 ± 3.83 b	33.00 ± 2.00 bc	54.00 ± 7.66 b	62.00 ± 5.16 a	147.89 (134.61 – 165.84)	373.76 (302.44 – 509.18)
Fruit Peel / Ethyl acetate	0.00 ± 0.00	8.00 ± 3.27 bc	19.00 ± 3.83 b	38.00 ± 2.31 c	60.00 ± 5.66 b	73.00 ± 3.83 b	128.72 (118.50 – 141.22)	304.55 (256.26 – 387.95)
Fruit Peel / Methanol	0.00 ± 0.00	11.00 ± 2.00 c	19.00 ± 2.00 b	48.00 ± 3.27 d	72.00 ± 5.66 c	80.00 ± 3.27 b	111.59 (103.48 – 120.65)	244.89 (213.31 – 294.92)
Temephos 1 mgL^{-1} 100.00 ± 0.00								

Note: Mean of 4 replications followed by the different lower-case letters within the same column (treatment concentration only) represent statistically significantly differences at $p < 0.05$ using one-way ANOVA and followed by LSD test. LC_{50}, lethal concentration revealing 50% mortality; LC_{90}, lethal concentration revealing 90% mortality; LCL, lower confidence limits; and UCL, upper confidence limits.

Table 7: LC_{50} and LC_{90} (mgL^{-1}) values of different solvent extracts of *C. fistula* against *Ae. aegypti* larvae and pupae (laboratory and field strains) after 24 h of exposure.

C. fistula extracts	*A. aegypti* (laboratory strain)				*A. aegypti* (field strain)			
	Larval stage		Pupal stage		Larval stage		Pupal stage	
	LC_{50} (mgL^{-1})	LC_{90} (mgL^{-1})	LC_{50} (mgL^{-1})	LC_{90} (mgL^{-1})	LC_{50} (mgL^{-1})	LC_{90} (mgL^{-1})	LC_{50} (mgL^{-1})	LC_{90} (mgL^{-1})
Seed / Ethyl acetate	124.36	270.13	161.61	366.57	153.29	356.82	185.87	429.01
Seed / Methanol	110.18	215.29	148.63	318.78	141.55	314.57	177.19	418.59
Fruit Pulp / Ethyl acetate	107.03	209.75	130.99	297.43	132.55	290.78	154.93	361.44

Fruit Pulp / Methanol	101.34	191.96	122.64	298.64	117.14	254.60	147.89	373.76
Fruit Peel / Ethyl acetate	100.88	198.41	113.46	247.96	115.18	240.24	128.72	304.55
Fruit Peel / Methanol	92.80	173.83	102.12	213.89	100.47	207.70	111.59	244.89

Adulticide Activity

Adult *Ae. aegypti* from both laboratory and field strains showed 100% mortality in the positive control (0.05% deltamethrin) and no mortality in the negative control (DMSO). Among all extracts tested, the methanolic fruit peel extract exhibited the highest adulticidal activity, causing 44.00% mortality in the laboratory strain at 200 mg/L, followed by fruit pulp (31.00%) and seed extracts (17.00%). A similar trend was observed with ethyl acetate extracts, where fruit peel again produced the greatest mortality (28.00%), compared with fruit pulp (20.00%) and seed (9.00%) (Fig.1A). Importantly, no recovery was observed during the post-exposure holding period. The overall effectiveness across both solvents consistently followed the order: fruit peel > fruit pulp > seed (Fig.1B). Adult mosquitoes from the laboratory strain were more susceptible than those from the field strain, aligning with the patterns observed in the larval and pupal assays.

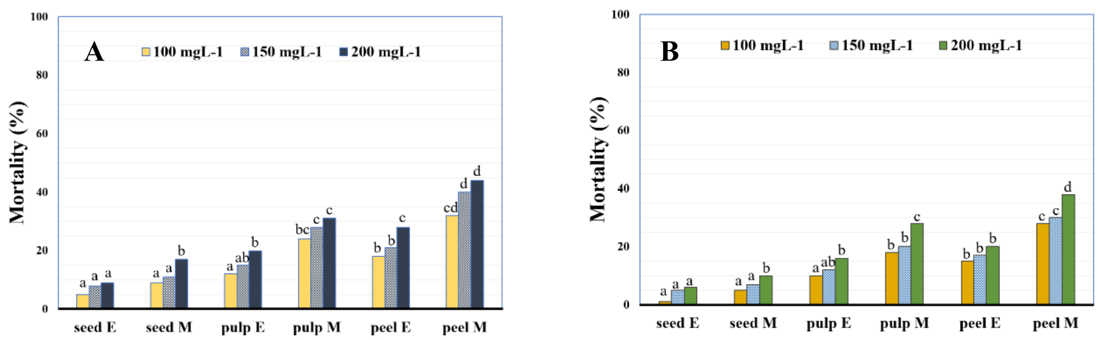

Figure 1: Mortality percentage of *Ae. aegypti* adults (A: laboratory strain, B: field strain) treated with *C. fistula* extracts at 100, 150, and 200 mg/L. E: ethyl acetate; M: methanol. Different letters indicate statistically significant differences ($p < 0.05$).

DISCUSSION

Phytochemical Analysis

Phytochemical screening revealed that anthraquinones were the dominant compounds in both methanol and ethyl acetate extracts of *C. fistula* pods. Notably, seven anthraquinones were identified, including physcion-8-O-β-D-glucoside, sennoside, aloin, chrysophanol-8-O-D-glucoside, aloe-emodin, emodin-8-O-β-D-glucoside, and most remarkably, 1,8-dihydroxy-3-anthraquinone (Danthron).

It is particularly novel to identify Danthron only in the fruit peel since no prior research has found it in this part of *C. fistula*. This could be linked to the biological role of the fruit peel as a protective barrier, which commonly leads to the accumulation of bioactive secondary metabolites to protect against herbivores and environmental stressors (Diverkar et al., 2022). In contrast, the seed, primarily involved in storage and germination, exhibited the lowest levels of such bioactivity. These findings suggest a tissue-specific distribution of anthraquinones, with the fruit peel serving as a promising source of potent insecticidal compounds.

Larvicidal and Pupicidal Activities

Environmental conditions in the bioassays remained within the optimal range for *Ae. aegypti* development (25–27°C and 60–70% humidity), confirming that observed mortality resulted solely from treatment effects. The crude ethyl acetate and methanolic extracts of *C. fistula* seed, fruit pulp, and fruit peel exhibited significant larvicidal and pupicidal efficacy, with methanolic extracts—particularly from the fruit peel—demonstrating higher effectiveness in both laboratory and field strains. Mortality increased in a concentration-dependent manner, with methanolic extracts continuously surpassing ethyl acetate extracts, possibly due to their superior capacity to extract polar bioactive chemicals, including emodin, aloe emodin, danthron, and chrysophanol-8-O glucoside. (Abutaha et al., 2020; Govindarajan, 2009).

Building on these findings, larvae were more susceptible than pupae, which aligns with their feeding behavior and greater exposure surface, whereas pupae possess reduced activity and a more protective cuticle (Shaalan et al., 2005). Field strains also exhibited lower sensitivity than laboratory strains, likely reflecting prior exposure to environmental stressors and potential chemical insecticides, contributing to physiological resistance (Moyes et al., 2017).

Further chemical analysis supported these biological observations. LC-MS analysis confirmed multiple anthraquinones in *C. fistula* extracts. Among these, danthron and emodin are known to disrupt mitochondrial activity, induce oxidative stress, and damage larval midgut tissues (David et al., 2000; Ray et al., 1999; Kamaraj et al., 2011). The presence of danthron exclusively in the fruit pulp and peel—and its absence in seeds—corresponds with the consistently higher larvicidal and pupicidal efficacy observed in these two plant parts. The fruit peel, which contained the most diverse anthraquinone profile, appears to offer the greatest potential due to the combined and possibly synergistic actions of danthron, emodin, and aloe emodin. These results indicate how important it is to focus on certain plant tissues, especially the fruit peel, when making efficient botanical treatments to control mosquitoes.

Adulticide Activity

The plant product of phytochemical, which is used as insecticides for killing adult mosquitoes. The findings of the current study reveal that the crude ethyl acetate and methanol extracts of *C. fistula* seed, fruit pulp, and fruit peel could be potential adulticidal against the dengue vector mosquitoes. The highest percentage mortality of *A. aegypti* laboratory and field strain adult were found in fruit peel methanolic extracts, which, at 200 mgL^{-1}, revealed 44.00 and 38.00 percent mortality, respectively.

Certain phytochemicals function as insecticides against mosquitoes in all stages, including adult, larval, and pupal stages; however, other phytochemicals are limited to use as adultucide, pupicide, or larvalicide. According to the current study's findings, *C. fistula* dried fruit peel extract effectively against *Ae. aegypti* larvae and pupae as an environmentally friendly insecticide. Because *Aedes* larvae, and pupae are stationary targets, controlling mosquitoes in their aquatic habitat is more successful than targeting adult *Aedes* mosquitoes.

These phytochemicals mainly impact the midgut epithelium, with secondary effects on the gastric caeca and malpighian tubules in mosquito larvae (David *et al.* 2000; Rey *et al.*1999). Lesions revealing edema, swelling, and distortion or elongation of the epithelial cells were seen in the larva's midgut (Jiraungkoorskul & Jiraungkoorskul 2015). Moreover, Danthron action is strongly suppressed DNA-binding activity of the aryl hydrocarbon receptor (AhR) (Fukuda *et al*. 2009).

Several previous studies have investigated the adulticidal effects of plant-based compounds, especially focusing on hexane, methanol, and essential oil extracts. For example, Abutaha et al. (2020) reported that the hexane–methanol soluble fraction (HMSF) from *C. fistula* fruit exhibited potent adulticidal activity against *Culex pipiens*, inducing 100% mortality at 80–100 mg/test tube within 30 minutes. These findings align with the current study, which demonstrates that methanolic and ethyl acetate extracts of *C. fistula* fruit peel, pulp, and seed possess notable adulticidal activity against *Ae. aegypti*. However, the efficacy was lower compared to larvicidal and pupicidal activity, likely due to differences in physiological barriers and detoxification mechanisms in adult mosquitoes.

CONCLUSION

This study highlights the significant larvicidal and pupicidal potential of *C. fistula* pod extracts, particularly those derived from the fruit peel using methanol. LC-MS profiling confirmed the presence of key anthraquinones, including danthron, emodin, and aloe-emodin, known for their potent bioactivity against mosquito larvae and pupae. The differential distribution of these compounds among plant parts, with fruit peel showing the richest profile, underscores the importance of tissue-specific phytochemical extraction. While the extracts exhibited slightly adulticidal effects, the efficacy was substantially lower compared to immature stages. This disparity is attributed to physiological differences, including the adult mosquito's more developed cuticle barrier and enhanced detoxification mechanisms. In contrast, larvae and pupae possess more permeable integuments and vulnerable target sites, making them more susceptible to phytochemical disruption.

Notably, danthron was identified in the fruit peel for the first time, highlighting its potential as a novel larvicidal agent. These findings support the use of *C. fistula* fruit peel extract as a promising, environmentally safe alternative for mosquito control, aligning with the growing need for plant-based vector management strategies.

ETHICAL CONSIDERATIONS

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTIONS

Prachumporn Lauprasert: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing-original draft, and Writing-review & editing.
Komsorn Lauprasert: Data curation, Resources, Validation, Writing-original draft, and Writing-review & editing.
Kongkaew Yaoup: Conceptualization, Methodology, and Resources.

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