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Potent larvicidal activities of *Capsicum frutescens*(L.) fruit ethanolic and partially purified extracts against *Aedes aegypti*(L.) and *Aedes albopictus*(S.)

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ABSTRACT

Dengue is one of the most rapidly spreading vector-borne diseases in the world. It affects many countries, especially the ones in the tropical areas like Philippines, Cambodia, Malaysia, India, Indonesia, Thailand, Brazil, Argentina, Eastern Africa and Nigeria, just to name a few. Methods for exterminating the adult mosquitoes involve fumigation, bug zappers, mechanical pest control, and use of adulticides; all of which have been shown to be either inefficient, toxic or both. To circumvent the problems posed by the other methods, the usage of botanical or plant derive products can be done instead. In this study, the larvicidal activities of ethanolicC. frutescent fruit extract and its partially purified fraction – using normal phase column chromatography – were tested against two primary dengue vectors, A. aegyptiand A. albopictus. The LC_{50} and LC_{90} after 24 and 48 hours of exposure were approximated using probit analysis. It was found out that the partially purified fraction was more active than the crude extract, by having higher average percent mortality and lower LC_{50} and LC_{90}. Phytochemical screening of the extract and fraction showed positive for tannins, alkaloids, coumarins, indoles and steroids in both and flavonoids in the crude extract only.

INTRODUCTION

Dengue is one of the most rapidly spreading vector-borne diseases in the world. It affects many countries, especially the ones in the tropical areas like Philippines, Cambodia, Malaysia, India, Indonesia, Thailand, Brazil, Argentina, Eastern Africa and Nigeria, just to name a few. For fifty years prior to 2007, incidence of dengue has increased 30-fold due to increasing geographic expansion, as well as from rural areas to urban settings. It is a vector-borne disease, transmitted primarily through the bites of infected Aedesaegypti mosquitoes. Besides Aedesaegypti mosquitoes, other Aedes mosquitoes have been known to spread dengue outbreaks, like Aedesalbopictus, Aedespolynesiensis, and Aedesscutellaris complex. Each of these Aedes mosquitoes has their own ecology, behavior and distribution **[1]**.Methods for exterminating the adult mosquitoes involve fumigation, bug zappers, mechanical pest control, and use of adulticides; all of which have been shown to be either inefficient, toxic or both. A better way of controlling mosquito populations – and in turn, control dengue spread – is by targeting the larvae instead of the adults. Lavicidal agents are commonly bacterial (e.g. Btlarvicides) or synthetic (e.g. methoprene, pyriproxyfen) in nature. Efficient and effective these agents may be, they still have certain issues regarding quality control and **[1]**. To circumvent the problems posed by the other methods, the usage of botanical or plant derive products can be done instead.

Capsicum is a genus of shrubs belonging to the family *Solanaceae*. Although its species are native to the Americas, they have now been cultivated worldwide. The fruits of *Capscimfrutescens* (L.) contain hot flavors, primarily due to the presence of alkaloid compounds called capsaicinoids, especially capsaicin and dihydrocapsaicin**[2]**.

Besides its use as a food spice, *C. frutescens*fruits have also been found to possess larvicidal and insecticidal properties. *Capsicum* extracts have been shown to be a repellent against stored product beetles like *Sitophiluszeamais*Motschulsky (Coleoptera: Curculinidae) and *Triboliumcastaneum*(Herbist) (Coleoptera: Tenebriouidae) **[3]**. It also has been shown to be insecticidal against rice grain pest, *Sitotrogacerealella***[4]**and Alfalfaweevil larvae, *Hyperbrunneipennis***[5]**.

In this study, the larvicidal activities of ethanolic*C. frutescent* fruit extract and its partially purified fraction – using normal phase column chromatography – were tested against two primary dengue vectors, *A. aegypti*and *A. albopictus*. To compare, the LC_{50} and LC_{90} values after 24 and 48 hours of exposure were approximated using probit analysis. Phytochemical screening for flavonoids, steroids, tannins, alkaloid, anthraquinone, anthrones, coumarins and indoles via spray tests.

MATERIALS AND METHODS

Chemicals and Reagents

95% Ethanol, Hexane, Ethyl Acetate (ChemLineInc.,Philippines), Dimethyl Sulfoxide (DMSO), TLC Silica Gel 60, Silca Gel G60 (Merck Inc., Philippines)

Larvae Sampling

*A. aegypti*and *A. albopictus*3rd instar larvae were obtained from the Research Institute for Tropical Medicine, Muntinlupa, Philippines.

Plant Sampling and Preparation

Capsicum frutescens(L.) fruits were purchased from local markets in Isabela, Philippines which were collected during August 2014. The plant samples were authenticated by Mr. DaniloTandang from the Botany Division, National Museum, Philippines. The samples were air dried under shade for two weeks, then ground until a coarse powder was obtained.

Extraction

Thirty grams of the powder was extracted with 95% ethanol in a 1 g plant material:10 mL solvent ratio. The mixture was left to soak for 48-72 hours, then filtered. The filtrate was concentrated with a rotary evaporator and then completely dried in a water bath at 45°C.

Partial Purification

The extract was subjected to normal phase column chromatography in order to separate its components. Silica gel G60 was used as the stationary phase whereas the varying solvent combinations of increasing polarity were used as the mobile phases. The slurry used to pack the column was composed of silica gel and hexane. The packed column used was 3 cm in diameter and 36 cm in height for all runs. The samples were prepared in an evaporating dish by adsorbing 1.55 g of the crude extract to 3.9 grams of silica gel G60 and drying on a water bath at 40°C. Elution was done with solvent systems of gradually increasing polarity using hexane, ethyl acetate, methanol, deionized water and deionized water-acetic acid. The following ratios of 100% hexane, 50% hexane:50% ethyl acetate, 100% ethyl acetate, 80% ethyl acetate:20% methanol, 60% ethyl acetate:40% methanol, 40% ethyl acetate:60% methanol, 20% ethyl acetate:80% methanol, 100% methanol, 70% methanol:30% deionized water, 60% methanol:40% deionized water, 100% deionized water, 80% deionized water:20% acetic acid, and 70% deionized water:30% acetic acid were sequentially used in the elution process. Measured volumes (200 mL) of each solvent combination was gradually poured into the column. Fifteen (15) mL eluents were collected and were pooled using a solvent system of 5 chloroform:1 methanol. Pooled fractions were then concentrated using a rotary evaporator at 40°C and then evaporated at 40°C to remove the solvent.

Larvicidal Screening of Fractions

Larvicidal assay was based on the Guidelines for Laboratory and Field Testing of Mosquito Larvicides**[6]** with slight modifications. The fractions were tested against *A. aegypti* and *A. albopictus* larvae separately. Prior to

assaying, larvae were allowed to acclimatize in double distilled dechlorinated water (dddH2O) for 2-3 hours. Glass assay cups containing 60 mL 1% DMSO were prepared as negative controls. The fractions were reconstituted in 1% DMSO to 300 ppm. There were 3 replicates for each treatment –fractions and negative controls. Batches of 15 third instar larvae were put into the assay cups containing 60 mL of 300 ppm reconstituted extracts and left for 48 hours. Throughout the assay, the ambient temperature was kept at 30±2 ℃. Dead larvae were counted after 24 and 48 hours. Larvae were counted as dead if they cannot be induced to move when the cups were shaken, or prodded with a needle or if they are moribund. The fraction with the highest mortality and the crude extract were subjected to dose-response assay.

Dose-Response Assay

Batches of 15 third instar larvae were transferred into glass assay cups containing 60 mL of treatments. For the active extract, the treatments were 200, 300, and 400 ppm extract while for the active fraction, the treatments were 100, 200, 300 and 400 ppm fraction. For each treatment, three replicates were performed. Throughout the assay, the ambient temperature was kept at 30±2 ℃. Dead larvae were counted after 24 and 48 hours of exposure. The LC_{50} and LC_{90} after 24 and 48 hours of exposure to the most active extract and its most active fraction against *A. aegpyti* and *A. albopictus* were determined using probit analysis.

Phytochemical Analysis

The extract and most active fraction were screened for the presence of flavonoids, steroids, tannins, alkaloid, anthraquinone, anthrones, coumarins and indoles via spray tests.

Statistical analysis

Corrected percent mortality was computed for both crude extract and fractions from Abbott's formula:

$$Corrected\ \%\ mortality = \frac{Mortality\%treatment - Mortality\%\ in\ control}{100 - Mortality\%\ in\ control} * 100 \quad (1)$$

And percent mortality was computed using the following formula:

$$Mortality\% = \frac{PercentSurvival_{control} - PercentSurvival_{treatment}}{PercentSurvival_{control}} * 100 \quad (2)$$

To compute for the LC_{50} and LC_{90}, probit analysis was performed using LDP Line software.

RESULTS AND DISCUSSION

The crude extract was partially purified using column chromatography; the result was five fractions: a hexane fraction (#1), ethyl acetate (#2), methanol (#3), aqueous (#4) and acetic acid-water (#5) fraction. These fractions were screened for larvicidal activity at 300 ppm, using 1% DMSO as negative control. Of these fractions, only fraction #2 exhibited maximum (100%) larvicidal activity against both species; the rest did not exhibit any activity. Since only fraction #2 exhibited larvicidal activity at 300 ppm, it was automatically chosen as the most active fraction. This fraction was subjected to dose-response assay and phytochemical screening.

*C. frutescens*ethanolic extract and the active fraction were subjected to dose-response assay to determine the LC_{50} and LC_{90} after 24 and 48 hours against *A. aegypti* and *A. albopictus*(Tables 1& 2). For both treatments, the increase in average percent mortality at increasing concentration indicates that the treatments are dose-responsive. Since for all concentrations, the average percent mortality for the active fraction is higher than the crude extract, it can be said that the active fraction has higher larvicidal activity than the crude extract.

Using the results of the dose-response assay, probit analysis was performed to approximate the LC_{50} and LC_{90} after 24 and 48 hours of both crude extract and active fraction (Table 3). For the crude extract against *A. aegypti*, the mean LC_{50} and LC_{90} after 24 hours were 231.59 ppm and 430.95 ppm, respectively while after 48 hours, they were 141.92 ppm and 346.74 ppm, respectively. For *A. albopictus*, the mean LC_{50} and LC_{90} after 24 hours were 300.20 ppm and 408.92 ppm, respectively while after 48 hours, they were 270.61 ppm and 474.60 ppm, respectively. Its most active fraction was also subjected to dose-response assay to determine the LC_{50} and LC_{90} after 24 and 48 hours of exposure against *A. aegypti* and *A. albopictus*. For *A. aegypti*, the LC_{50} and LC_{90} after 24 hours were 97.22 ppm and 181.13 ppm, respectively while after 48 hours, they were 66.02 ppm and 132.80 ppm, respectively. For *A.*

albopictus, the LC_{50} and LC_{90} after 24 hours were 41.74 ppm and 164.53 ppm, respectively while after 48 hours, they were 24.35 ppm and 83.78 ppm, respectively. Table 3 summarizes the results of the probit analysis, including the mean LC_{50} and LC_{90} values after 24 and 48 hours, fiducial limits, $\chi 2$ and r2 values.

Table 1. Results of the dose-response assay of ethanolic*C. frutescens* crude extract against *A. aegypti* and *A. albopictus*

Treatment	Species	Exposure (hrs)	Average percent mortality	Standard deviation
Negative control: 1% DMSO	*Ae. aegypti*	24	0%	0
		48	0%	0
	Ae. albopictus	24	0%	0
		48	0%	0
Crude extract 200 ppm	*Ae. aegypti*	24	37.78%	3.143
		48	68.89%	8.315
	Ae. albopictus	24	2.22%	3.143
		48	8.89%	3.143
Crude extract 300 ppm	*Ae. aegypti*	24	71.11%	6.285
		48	86.67%	5.443
	Ae. albopictus	24	57.78%	6.285
		48	73.33%	5.443
Crude extract 400 ppm	*Ae. aegypti*	24	86.67%	5.443
		48	95.56%	3.143
	Ae. albopictus	24	84.44%	3.143
		48	91.11%	3.143

Table *2*. Results of the dose-response assay of ethanolic*C. frutescens* crude extract (ethyl acetate fraction) against *A. aegypti* and *A. albopictus*

Treatment	Species	Exposure (hrs)	Average percent mortality	Standard deviation
Negative control: 1% DMSO	*Ae. aegypti*	24	0%	0
		48	0%	0
	Ae. albopictus	24	0%	0
		48	0%	0
Fraction #2 100 ppm	*Ae. aegypti*	24	53.33%	0.000
		48	77.78%	3.143
	Ae. albopictus	24	82.22%	3.143
		48	93.33%	0.000
Fraction #2 200 ppm	*Ae. aegypti*	24	91.11%	3.143
		48	97.78%	3.143
	Ae. albopictus	24	95.56%	3.143
		48	97.78%	3.143
Fraction #2 300 ppm	*Ae. aegypti*	24	100%	0.000
		48	100%	0.000
	Ae. albopictus	24	97.78%	3.143
		48	100%	0.000
Fraction #2 400 ppm	*Ae. aegypti*	24	100%	0
		48	100%	0
	Ae. albopictus	24	100%	0
		48	100%	0

The results of the dose-response assay indicate that the activity of *C. frutescens*ethanolic extract is dose-responsive; at increasing concentrations of the extract, the percent mortality also increases. The steep slopes of the probit regression models for *A. aegypti* after 24 and 48 hours, and for *A. albopictus* after 24 and 48 hours imply that even at low increases in extract concentration, a high larvae percent mortality can be observed. A $\chi 2$ value of less than 5.991 – the critical value for d.f. equals 2 – in all the regression models indicate high goodness-of-fit, while a coefficient of determination (r^2) value close to 1 imply that the results of the assays are reliable. Probit analysis of the most active fraction also indicate that the active fraction is dose-responsive. Again, the steep slopes indicate that even at low concentrations of the active fraction, a high larvae percent mortality can be observed. Since the $\chi 2$ value is also less than 5.991 and the r2 value is still close to 1, high goodness-of-fit and linearity of the regression models are observed. Since the LC_{50} and LC_{90} values for the active fraction are lower than those of the crude extract, it can be concluded that the active fraction is more potent than the crude extract. No overlaps in the fiducial limits for the LC_{50} and LC_{90} values except for the LC_{50} after 24 hours vs. *A. aegypti* imply that these values are significantly different.

Table *3*. Results of the probit analyses of ethanolic*C. frutescens* crude extract and fraction #2 against *A. aegypti* and *A. albopictus*

Treatment	Mosquito Species	Exposure	LC_{50}[1](LL, UL)[3]	LC_{90}[1](LL, UL)[3]	Slope ± SE	X^2*	r^2
*Capsicum frutescens*ethanolic extract	*A. aegypti*	24	231.5881 (192.9089, 259.7932)	430.9479 (366.6488, 603.8086)	4.7518±0.9815	0.0205	0.999
		48	141.9191 (52.6713, 188,5988)	346.7425 (292.7324, 514.5759)	3.3034±0.9869	0.0007	0.993
	A. albopictus	24	300.2024 (280.5081, 320.3958)	408.921 (375.4258, 467.281)	9.5481 ± 1.3413	2.3576	0.974
		48	270.6146 (252.1678, 288.2887)	374.6019 (346.4148, 418.9785)	9.0753 ± 1.1284	1.9610	0.974
*Capsicum frutescens*ethanolicextract – fraction #2	*A. aegypti*	24	97.2225 (76.341, 113.3585)	181.1339 (155.7739, 228.5227)	4.7426 ± 0.8384	0.7031	0.943
		48	66.0239 (29.0088, 86.3972)	132.8026 (108.3948, 178.3831)	4.2227 ± 1.2109	0.0848	0.961
	A. albopictus	24	41.7358[4]	164.5353[4]	2.1512 ± 0.7327	3.6250	0.895
		48	24.3486[4]	83.7848[4]	2.3880 ±	0.3828	0.907

1 mg extract/L
[3]LL=lower fiducial limit; UL = upper fiducial limit
[4]The LL and UL cannot be computed because their g (index of significance of potency) > 0.04.
**df=2*

Phytochemical screening for the presence of flavonoids, steroids, tannins, alkaloids, anthraquinones, anthrones, coumarins and indoles in the extract and active fraction was performed, the results of which is presented in Table 4. Alkaloids **[7]**, tannins **[8]**, and indoles**[9]** have been shown to possess toxic activities against insects; hence, their presence in the extracts can be the cause of their larvicidal activity. Phytochemical screening of the active fraction also showed tannins, alkaloids, coumarins, indoles and steroids. The presence of alkaloids imply the presence of capsaicinoid compounds, especially capsaicin – a known pest toxicant that causes metabolic disruption, membrane damage and nervous system dysfunction **[10]**.

Table 4. Phytochemical screening summary

Phytochemical	*C. frutescensethanolic*	*C. frutescensethanolic – fraction #2*
Flavonoid	+	-
Tannin	+++	+++
Alkaloid	+	+
Anthraquinone	-	-
Anthrone	-	-
Coumarin	+	+
Indole	++	++
Steroid	++	++

'-' No reaction; '+' weak intensity reaction; '++' intense reaction; '+++' strong intensity reaction

The larvicidal activity of *C. frutescens* leaf and fruit methanol extracts against second and third instar *A. aegypti* larvae have been demonstrated by **[11]**. At 100 mg/mL, the percent moralities were 92% and 99% after 24 hours of the leaf and fruit extracts, respectively, against second instar larvae and 99% and 99.3% after 48 hours of the leaf and fruit extracts, respectively. On the other hand, at 100 mg/mL, the percent mortalities were 41.3% and 91.6% after 24 hours of the leaf and fruit extracts, respectively, against third instar larvae and 66% and 99.6% after 48 hours of the leaf and fruit extracts, respectively. They also performed a preliminary phytochemical screening and found the presence of tannins, alkaloids, steroids and glycosides. The larvicidal activity of the fruit ethanol extract was also tested against *Anopheles stephensi* and *Culexquinquefasciatus***[12]**. The LC_{50}, LC_{90} and LC_{99} values against *C.quinquefasciatus*were 0.0097%, 0.022% and 0.044% oleoresin solution, respectively, while against *An.stephensi*, they were 0.011%, 0.027% and 0.057% oleoresin solution, respectively.

CONCLUSION

After dose response assay of the *C. frutescens*ethanolic extract, the LC_{50} and LC_{90} values were determined. The LC_{50} and LC_{90} values were 231.59 and 430.95 ppm, respectively at 24 hours and 141.92 and 346.74 ppm, respectively at 48 hours for *A. aegypti*. The LC_{50} and LC_{90} were 300.20 and 408.92 ppm, respectively at 24 hours and 270.61 and 374.60 ppm, respectively at 48 hours for *A. albopictus*. Partial purification successfully obtained the most active fraction of *C. frutescens*. After dose-response assay, the LC_{50} and LC_{90} values of this active fraction were determined. The LC_{50} and LC_{90} values were 97.22 and 181.13 ppm, respectively at 24 hours and 66.02 and 132.80 ppm, respectively at 48 hours for *A. aegypti*. The LC_{50} and LC_{90} values were 41.74 and 164.54 ppm, respectively at 24 hours and 24.35 and 83.78 ppm, respectively at 48 hours for *A. albopictus*. Phytochemical screening of the crude extract and active fraction revealed the presence of tannins, alkaloids, coumarins, indoles and steroids and flavonoids in the crude extract.

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REFERENCES

[1] World Health Organization - The Special Programme for Reaserch and Training in Tropical Diseases; TDR Results 2013 Report. Geneva, Switzerland, **2013**
[2] V.S. Govindarajan, M.N. Sathyanarayana. *Food SciNutr*, **1991**, 29, 435-74.
[3] S.H. Ho, Y. Ma, H.T.W. Tan, H. Halid. *Proceedings of the symposium on pest management for stored food and feed*(BIOTROP), **1997**, 59, 209-15.
[4] A.M. Prakash, J. Rao. *Entomon*, **2006**, 31, 1-8.
[5] M.A. Al-Doghairi, E.E. Hag. *Pak J BiolSci*,**2003**, 6, 777-781.
[6] World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. World Health Organization Press, **2005**
[7] Z.L. Liu, Q.Z. Liuo, S.S. Du., Z.W. Deng. *Paras Res*, **2012**, 111(3), 2923-2929.
[8] H.H.G. da Silva, L.G. da Silva, R.M.G. dos Santos, E.R. Rodrigues, C.N. Elias. *Rev. Soc., Bra. Med. Trop.*, **2004**, 396-399.
[9] V.L. Ranganatha, A.B. Begum, T. Prashanth, H.D. Gurupadaswamy, S.K. Madhu, S. Shivakumar, S., S.A. Khanum. *Drug Intervention Today*, **2013**, 5(4), 1-6.
[10] National Pesticide Center. Capsaicin technical fact sheet,**n.d.**. Retrieved from: http://npic.orst.edu/factsheets/Capsaicintech.pdf
[11] K.S. Vinayaka, T.R. PrashithKekuda, K.C. Nandino, M.N. Rakshitha, MartisRamya, J. Shruthi, G.R. Nagashree, B. Anitha. *Der Pharm. Lett.*,**2010**, 2(4), 172-176.
[12] A.P. Madhumathy, Ali-Ashraf Aivazi, V.A. Vijayan. *J. Vect. Borne. Dis*, **2007**, 44, 223-226.