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Ovicidal, larvicidal, and adulticidal activities of *Citrus grandis* (L.) Osbeck against dengue vector, *Aedes aegypti* (L.)

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Recent studies regarding the harmful effects of synthetic larvicides initiated the need to investigate for unconventional measures that are environmentally-safe and target-specific against *Aedes aegypti* larvae. Thus, the main objective of the study was to evaluate the ovicidal, larvicidal, and adulticidal toxicity of the hexane extract of *Citrus grandis* (L.) Osbeck peels against dengue vector, *A. aegypti.* Results revealed that the hexane extract of *C. grandis* peel from Davao exhibited the highest lethal concentration against 3rd and 4th instar larvae with an LC_{50} and LC_{90} of 1.11 mg/L and 3.32 mg/L, respectively. Moreover, the knockdown effect of the hexane extract of *C. grandis* peels on adult female *A. aegypti* produced 50 % knockdown within 11.50 min and 90 % knockdown within 28.79 min. The test mosquitoes' mortality was 100 % after 24 h. Lastly, the ovicidal activity of the hexane extract against the eggs of *A. aegypti* exhibited an LC_{50} of 13.84 mg/L and LC_{90} of 25.30 mg/L. The remarkable effects exhibited by *C. grandis* peels indicate its great potential to be a more sustainable and environmentally-safe plant-based control for the proliferation of the dengue vector, *A. aegypti.*

Keywords: Adulticidal, *Aedes aegypti, Citrus grandis* (L.) Osbeck, Dengue vector, Larvicidal, Ovicidal.
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Introduction

Citrus grandis (L.) Osbeck, commonly known in the Philippines as *Suha*, is considered to be the largest citrus fruit and is actually known in the western world primarily as the principal progenitor of the grapefruit. It is also known as pummelo, pomelo, grapefruit, shaddock and forbidden fruit[1,2]. *C. grandis* is a small tree, 5 to 12 M in height, with a large fruit that has a thick rind and yellow to pink, sweet or acrid pulp. It is found throughout the Philippines in settled areas and is usually planted. It is native to Southeastern Asia and all of Malaysia[2].

In the Philippines, reports have shown that *C. grandis* leaves are locally used in aromatic baths. The leaves, flowers, and pericarps are used as a sedative in nervous affections in the form of a decoction. Boiled *C. grandis* leaves are employed hot on painful swellings in Malaya It is also reported that *C. grandis* is used in ulcers, epilepsy, chorea, and convulsive coughs. There are also reports that the outer part of the rind makes an excellent cordial, which is also employed by the Chinese in dyspepsia and coughs. The seeds, also known as pips have somewhat the same properties and are given for lumbago[1].

Aedes aegypti (L.) (Diptera: Culicidae) is a mosquito that acts as the main vector in the transmission of yellow fever and dengue fever viruses, and feeds almost exclusively on humans[3,4]. These diseases are endemic in developing countries of Africa, Latin America and Asia[5]. The incidences of dengue have grown dramatically around the world in recent decades. One recent estimate indicates 390 million dengue infections per year (95 % credible interval 284–528 million), of which 96 million (67–136 million) manifest clinically (with any severity of disease)[6]. These are the reasons behind the efforts of many research institutions to find a solution in controlling the vector of dengue. The most widely used mosquito control is the chemical method because of the prompt results brought by this technique[7]. However, as a result of increased resistance of insects and pests to chemical pesticides, stricter environmental policies, awareness among the consumers and rising industrial R&D cost of synthetic insecticides, there has been a significant interest for the use of natural products as effective insecticides[8].

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The focus of the present investigation was on the study of *C. grandis* as a potential vector control measure against *A. aegypti* that is acceptable to the public, cost-effective, and more importantly safe for the environment. The objective of the study was to evaluate the ovicidal, larvicidal, and adulticidal toxicity of the extract from *C. grandis* peels collected from different localities in the Philippines.

Materials and Methods

Botanical material

The fruits of *C. grandis* were collected from five different provinces in the Philippines namely: Benguet (Cordillera Administrative Region); Antique (Region VI); Palawan (Region IV-B); Davao (Region XI); and South Cotabato (Region XII). Benguet and Iloilo have a Type I climate which means there are two pronounced season: dry from November to April and wet for the rest of the year. Davao and South Cotabato have a Type IV climate, which means rainfall is more or less evenly distributed throughout the year while Palawan and Antique have a Type III climate which means seasons are not very pronounced, relatively dry from November to April, and wet for the rest of the year. Specimen tags or labels were placed in each plant. Herbarium specimens of the plants collected were prepared and sent to the National Museum of the Philippines for identification and authentication.

Sample preparation

The *C. grandis* peels were oven-dried (Hereus, Model T5600) to moisture content below 10 %. The dried material was ground using Wiley mill (Thomas Scientific, Model 3379-K05-99). About 100 g of ground plant material was macerated with 400 mL n-hexane (RCI Labscan Bangkok, Thailand) for 48 h. Then, the mixture was filtered and the filtrate was concentrated under vacuum at 55 °C to a syrupy consistency. This was further evaporated in a water bath (Biobase Model: SY-2L8H) at the same temperature until completely dried.

Rearing of *Aedes aegypti* eggs

The *A. aegypti* mosquito eggs and larvae used in the study were reared in the Insectary of Standards and Testing Division, Industrial Technology Development Institute, Department of Science and Technology, Taguig City, Metro Manila, Philippines at a laboratory condition of 27±2 ^{o}C temperature and 80±10 % relative humidity. About 3-5 days old batches of eggs were used for ovicidal testing. Some mosquito eggs were hatched in dechlorinated tap water that served as the culture medium at 28±2 °C and a photoperiod of 12 h light followed by 12 h dark (12 L:12 D). Appropriate amount of yeast was added to enhance the growth of the larvae. The 3rd and early 4th instar larvae were used in the larvicidal bioassay.

Ovicidal and larvicidal bioassay

The ovicidal and larvicidal toxicity of the extract were evaluated using a modified method of the WHO guidelines for laboratory and field testing of mosquito larvicides[9]. Larvicidal bioassay was done with a chemical larvicide, Abate 1SG and a biolarvicide, PhBigR for comparative evaluation. Dechlorinated water was used as the negative control.

Knock-down test (Close-cylinder method)

The adulticidal toxicity of the extract was tested following the WHO guidelines for testing mosquito adulticides[10].

Data analysis

Data from all replicates were pooled for analysis. LC_{50}, LC_{90}, and relative median potency values were calculated from a log dosage–probit mortality regression line using SPSS for Windows. Standard deviation of the means of LC_{50} values was calculated.

Results

Result of the larvicidal bioassay of the hexane extract of *C. grandis* peels collected from different localities is shown in Fig. 1. The *C. grandis* peels from Davao showed the lowest lethal concentration (LC_{50} = 1.11 mg/L, LC_{90} = 3.32 mg/L) followed by the one from Benguet (LC_{50} = 12.85 mg/L, LC_{90} = 29.25 mg/L). This was followed by the hexane extract from Antique, Cotabato and Palawan. Comparative evaluation of the larvicidal activity was done with chemical larvicide Abate 1SG and biolarvicide, PhBigR. The Abate 1SG showed lower lethal concentration (LC_{50} = 0.06 mg/L, LC_{90} = 0.11 mg/L) than the *C. grandis* hexane extract while PhBigR (Fig. 1) showed higher LC_{50} (1.71 mg/L) and LC_{90} (8.41 mg/L) values.

The relative median potency estimates of the larvicidal activity of *C. grandis* from different localities are shown in Table 1. *C. grandis* from Davao was the most potent followed by Benguet and Antique. The relative median potency is the ratio of the LC_{50} of *C. grandis* from different localities to the LC_{50} of the commercially available larvicides in the Philippines.

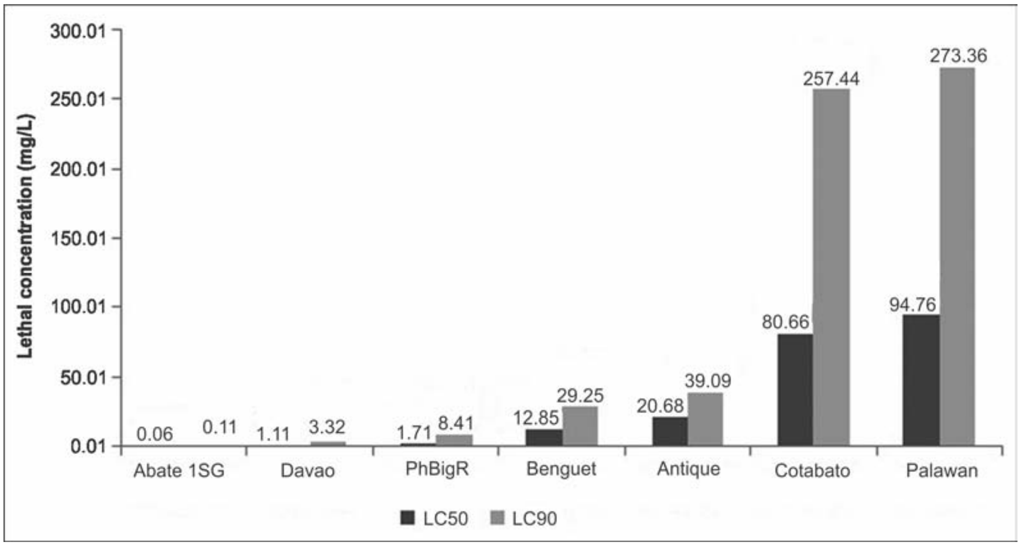

Fig. 1—Lethal concentrations of the hexane extract of *Citrus grandis* peels from different localities against 3rd and 4th instar larvae of *Aedes aegypti* against the chemical larvicide (Abate 1SG) and biolarvicide (PhBigR)

Table 1—Relative median potency of the larvicidal activity of *Citrus grandis* to commercially available larvicides (Abate 1SG and PhBigR)

C. grandis source	Relative median potency to Abate 1SG (95 % CI)	Relative median potency to PhBigR (95 % CI)
Davao	0.055 (0.036 – 0.079)	1.76 (1.47 – 2.12)
Benguet	0.005 (0.002 – 0.010)	0.16 (0.12 – 0.21)
Antique	0.003 (0.001 – 0.006)	0.10 (0.07 – 0.14)
Cotabato	0.001 (0.000 – 0.0002)	0.024 (0.014 – 0.038)
Palawan	0.001 (0.000 – 0.002)	0.022 (0.012 – 0.035)

CI-Confidence interval

The Knockdown effect of the hexane extract on adult female *A. aegypti* mosquitoes showed that 50 % mosquito knockdown (KT_{50}) was produced within 11.50 min and 90 % knockdown (KT_{90}) within 28.79 min. The test mosquitos' mortality was 100 % after 24 h. The extract was also tested against *A. aegypti* eggs. After 72 h, egg hatching was recorded and the lethal concentration was calculated. The LC_{50} and LC_{90} toxicity elicited by the extract were 13.84 and 25.30 mg/L, respectively.

Discussion

More than 2000 plant species have been reported in the literature to have insecticidal properties[9]. Several Philippine plants have been found to have mosquitocidal properties[12-15]. To consider an extract bioactive, it should show lethal concentrations (LC_{50} and LC_{90}) extremely lower than 1000 mg/L[16]. The varying larvicidal activities of the peel extracts can be associated to the type of soil, climate and season of the source which can influence the concentration of plant bioactive components[17]. The efficacy of larvicidal activity of plant can vary depending on plant species, plant parts used, age of plant parts (young, mature or senescent) and solvent used during extraction[18]. The larvicidal activity of the hexane extract is comparable to Abate 1SG and higher than PhBigR, which highly signifies its potential as a means to control against *A. aegypti.* Natural pesticides from plants are said to be a promising tool especially for targeting mosquitoes in the larval stage[19].

C. grandis belongs to the family Rutaceae and members of this family are said to have potent larval, adulticidal, and repellent activities against different species of mosquitoes[20]. Still, there are no studies specifically on ovicidal, larvicidal and adulticidal bioactivities of the hexane extract of *C. grandis* peels against *A. aegypti* but there are several mosquitocidal studies on other plants belonging to the same family. The flavonoid compounds found in *Poncirus trifoliate* (Family Rutaceae) exhibited potent activity against larvae and eggs of *A. aegypti* eggs, where the lethal concentration LC_{50} and LC_{90} of the flavonoids ranged from 0.082 to 0.122 mg/L and 0.152 to 0.223 mg/L, respectively[21]. Thavanapong reported that the essential oil from *C. grandis* peels is active against the larvae of *Culex quinquefasciatus*, *C. tritaeniorhynchus* and *A. aegypti* at a concentration of 20 mg/L (0.02 mL/L)[22]. Another study on the non-polar leaf extracts of *C. aurantifolia* and *C. hystrix* against *A. aegypti* larvae showed that extracts of both the plants were effective as biolarvicide with *C. aurantifolia* exhibiting higher toxicity than *C. hystrix*[23]. *C. sinensis* leaves are proved to be reasonably larvicidal and irritant against *A. aegypti*[24].

Conclusion

Countries burdened by this disease depend on vector control and continue to explore alternative methods to fully terminate the spread of dengue. The remarkable toxic effects exhibited by *C. grandis* peels against the eggs, larvae, and adults of *A. aegypti* signifies its potential as a more effective, eco-friendly and target-specific control for the dengue vector. It is advisable to study extensively the mosquitocidal property of the plant by isolating and identifying the active components and then using them in field trials in order to assess their full potential as an alternative to chemical dengue vector control.

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