The Journal of Animal & Plant Sciences, 25(4): 2015, Page: 1187-1190
ISSN: 1018-7081

Short Communication

LARVICIDAL ACTIVITY OF *Garcinia mangostana* FRUIT WASTES AGAINST DENGUE VECTOR *Aedes aegypti*

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ABSTRACT

This study was conducted to investigate the effect of the ethanol and hexane extracts of the fruit wastes of *Garcnia mangostana* (mangosteen) on the Dengue vector *Aedes aegypti*.The fruit wastes (pericarp, crown and seeds) were solvent extracted separately. The larvicidal activity of the extracts was tested against the 3rd and 4th instars larvae of the *Aedes aegypti* following the World Health Organization bioassay method (2005). Characterization of the extracts was conducted by qualitative phytochemical screening. The ethanol pericarp extract of *G. mangostana* exhibited the highest toxicity among the other parts (crown and seeds) against *A. aegypti* larvae with an LC_{50} values of 4.84 mg/L and 6.19 mg/L and LC_{90} values of 14.55 mg/L and 28.71 mg/L while the hexane extract yielded an LC_{50} and LC_{90} of 27.61 mg/L and 67.27 mg/L, respectively. The hexane crown extract also yielded larvicidal activity with an LC_{50} and LC_{90} of 25.33 mg/L and 63.73 mg/L, respectively while the ethanol extract gave an LC_{50} of 63.00 mg/L and LC_{90} of 169.91 mg/L. Meanwhile, both ethanol and hexane extracts of the seeds did not exhibit any toxic effect up to 500 mg/L and 300 mg/L, respectively. Results suggest the potential of *G. mangostana* fruit wastes as a key source for the development of environment-friendly plant-based larvicides.

Key words: *Garcinia mangostana*, dengue vector, *Aedes aegypti*, larvicidal activity, phytochemical screening.

INTRODUCTION

Dengue is a world-wide arthropod-borne viral disease transmitted by *Aedes* mosquitoes, typically *Aedes aegypti* (Capeding*et al.* 2013).It is an acute infection that kills faster than AIDS. Treatment is mainly supportive and prevention method presently depends on vector control until a vaccine becomes available. Use of chemical control is said to be an effective way used generally in daily life (Pavela, 2009) but alarms environmental and human advocates since widespread use of synthetic insecticides has led to many negative consequences (Pavela 2008). This results in the increasing attention and consideration to natural products (Pirali-Kheirabadi and da Silva 2010). Green larvicides are now being considered because plants consist of several bioactive components that are known to be safe and biodegradable. Unlike the conventional insecticide which is based on single active ingredient, plant insecticides comprise botanical blends of bioactive chemical compounds which act concertedly on both behavioural and physiological processes (Ghosh *et al.* 2012). The decades of research about interactions between plants and insects revealed the potential use of plants in fundamental pest control programs (Kamaraj *et al.* 2010). One of the several plants with insecticidal properties is *Garcinia mangostana* or commonly known as mangosteen. *G. mangostana* originated from the Southeast Asia but can now also be found in many parts of the world. It is not only popular because of its pleasant taste but also because of its numerous medicinal properties (Ji *et al.* 2007). This leads us to our main focus which is on the research study of *G. mangostana* as a potential vector control measure against *A. aegypti* that is acceptable to the populace, cost effective and more importantly, safe for the environment. The use of natural alternatives such as plant extracts can provide the best option as environmentally safe natural larvicides. Therefore, the objectives of the study were to evaluate the toxicity of the ethanol and hexane extracts of the fruit wastes of *G. mangostana* against 3rd and 4th instars larvae of *A. aegypti* and to characterize the ethanol extract by qualitative phytochemical analysis.

MATERIALS AND METHODS

Botanical Material: The fruits of *G. mangostana* were collected in Davao, Philippines. The different fruit wastes namely, the pericarp, crown and seeds were processed separately for crude extraction and larvicidal bioassay. Herbarium specimen of the plants collected was prepared and was sent to the National Museum of the Philippines for necessary identification and authentication.

Sample Preparation: The *G. mangostana* fruit wastes were separated and air-dried to a moisture content of less than 10%. The plant materials were oven-dried at 60ºC for one week until the moisture content was less than 10% and were then ground with Wiley mill.About 300 grams of ground plant material were macerated with either 900 mL of EtOH and n-hexane for 48 hours. Then, the mixture was filtered with a coarse filter paper and the filtrate was concentrated under vacuum at 55ºC to a syrupy consistency. This was further evaporated in a water bath at 55ºC until completely dried.

Rearing of *Aedes aegypti* larvae: The eggs of *A. aegypti* were reared in the Entomology Laboratory of the Industrial Technology Development Institute – Department of Science and Technology, Taguig City, Metro Manila, Philippines. The egg rafts were kept in the basin containing tap water that served as the culture medium at 28±2ºC and a photoperiod of 12 h light followed by 12 h dark (12L: 12D).Dog biscuits (0.5g) and yeast (0.3g) were added to enhance the growth of the larvae. The 3rd and 4th instars larvae were used in the study.

Larvicidal Bioassay against 3rd and 4th instars larvae of *A. aegypti:* The larvicidal bioassay was conducted following the WHO method (World Health Organization, 2005).Batches of 20 third or fourth instars larvae were transferred by means of droppers to small disposable test vessels, each containing 100 mL of water. The appropriate volume of dilution was added to 100 mL water in the cups to obtain the desired target dosage, starting with the lowest concentration. The test containers were held at 28±2ºC and a photoperiod of 12 h light followed by 12 h dark (12L: 12D). Four or more replicates were set up for each concentration and an equal number of controls were set up simultaneously with tap water, to which 1 mL ethanol (or the organic solvent used) was added. Each test was run three times on different days. The results were recorded where the $LC_{50}$and LC_{90}values and slope and heterogeneity analysis were also noted.

Data analysis: Data from all replicates were pooled for analysis. $LC_{50}$and LC_{90} values were calculated from a log dosage–probit mortality regression line using computer software programs. Standard deviation of the means of LC_{50} values were calculated and recorded. A test series is valid if the relative standard deviation (or coefficient of variation) is less than 25% or if confidence limits of LC_{50} overlap (significant level at $P < 0.05$).

Phytochemical Analysis: The qualitative phytochemical analysis of the ethanol pericarp and crown extracts was performed (Guevara, 2005) to detect the presence of alkaloids, tannins, 2-deoxysugars, saponins, free fatty acids, unsaturated steroids, triterpenoids, flavonoids, leucoanthocyanins, and anthraquinones.

RESULTS AND DISCUSSION

Results of the larvicidal bioassay of the ethanol fruit wastes of *Garcinia mangostana* were shown in Figure 1.Results were expressed as lethal concentration (LC) of the larvicide for 50% and 90% mortality (LC_{50} and LC_{90}) of the mosquito larvae. The ethanol pericarp extract of *G. mangostana* showed an LC_{50} values of 4.84 mg/L and 6.19 mg/L (average = 5.52 mg/L) and LC_{90} values of 14.55 mg/L and 28.71 mg/L (average = 21.63 mg/L) (Figure 1). Meanwhile, the hexane extract showed an LC_{50} value of 27.61 mg/L and LC_{90} value of 67.27 mg/L. The ethanol crown extract yielded an LC_{50} of 63.00 mg/L and LC_{90} of 169.91 mg/L while the hexane extract exhibited an LC_{50} and LC_{90} of 25.33 mg/L and 63.73 mg/L, respectively. On the other hand, the ethanol and hexane extracts of *G. mangostana* seeds did not exhibit any toxic effect up to 500 mg/L and 300 mg/L, respectively (Figure 2).

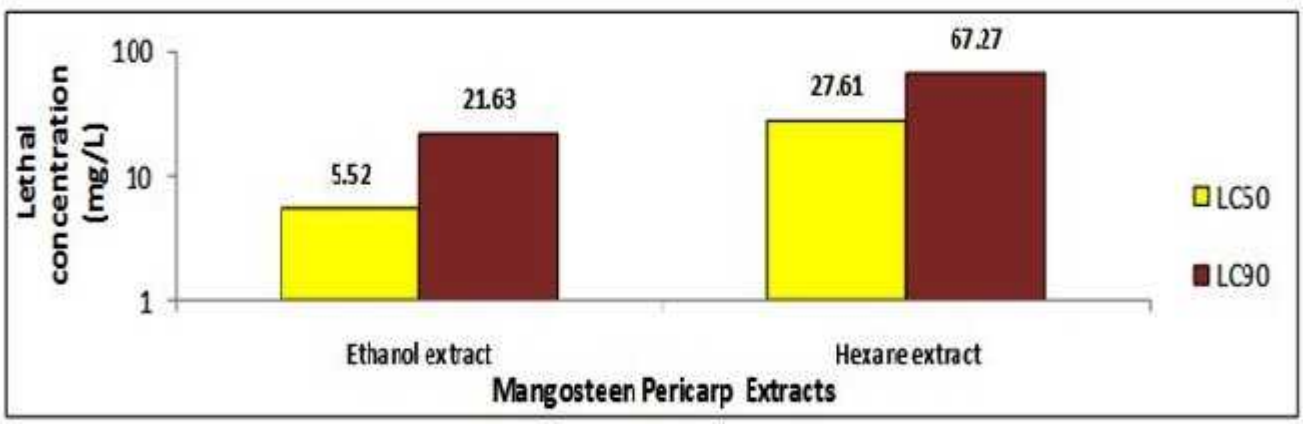

Figure 1. Comparison of average lethal concentration LC_{50} and LC_{90} of the ethanol and hexane extracts of *G. mangostana* pericarp.

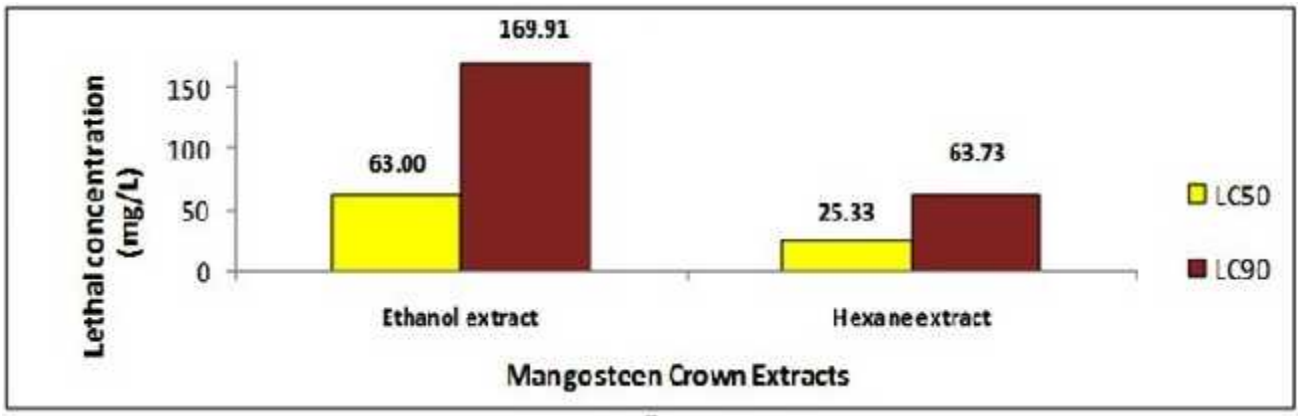

Figure 2.Comparison of lethal concentration LC_{50} and LC_{90} of the ethanol and hexane extracts of *G. mangostana* crown.

People have used different plants, products and secondary metabolites of plant origin in pest control since early times (Ghosh *et al.* 2012). That is why several studies have been dedicated on testing natural products against mosquitoes specifically, *A. aegypti* which produced varying results (de Morais *et al.* 2007; Gautam *et al.* 2013;Sutthanont *et al.* 2010). Several Philippine plants have been found to have various medicinal properties such as larvicidal properties (Thein *et al.* 2013; Monzon RB, *et al.*1994; Gutierrez *et al.* 2014; Torres *et al.* 2014).

In the present study, hexane and ethanol extracts of the fruit wastes such as the pericarp, crown and seeds of *G. mangostana* were subjected to larvicidal toxicity assay. The plant parts, specifically the pericarp and the crown that were studied showed great toxicities against *A. aegypti*. One literature reported *α*-mangostin, a novel insecticide that is toxic to larvae of several mosquito species including 3rd instar larvae of *A. aegypti* (Larson *et al.* 2010). The extracts were considered to be bioactive since they showed lethal concentrations (LC_{50} and LC_{90}) extremely lower than 1000 mg/L (de Morais *et al.* 2007). The solvent used can also contribute to the variation since it has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents used (Ghosh *et al.* 2012). The ethanol pericarp extract of *G. mangostana* exhibited higher toxicity than the hexane extract, while the hexane crown extract exhibited otherwise. Both ethanol and hexane extracts of the seeds did not show larvicidal activity up to 500 mg/L and 300 mg/L, respectively.

Phytochemical analysis of the ethanol pericarp extract of *G. mangostana* showed presence of alkaloids, saponins, fats and oils, 2-deoxysugars, leucoanthocyanins, flavonoids, hydrolyzable tannins, unsaturated steroids and triterpenoids. However, free fatty acids and anthraquinones were found not present. On the other hand, the ethanol extract of *G. mangostana* crown showed presence of saponins, fats and oils, 2-deoxysugars, leucoanthocyanins, flavonoids, condensed tannins, unsaturated steroids and triterpenoids and free fatty acids but indicated absence of alkaloids and anthraquinones (Table 1). It is said that several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Shaalan *et al*. 2005).

Table 1. Results of the qualitative phytochemical analysis of the ethanol extract of *G. mangostana* pericarp and crown.

Phytochemical	*G. mangostana* pericarp	*G. mangostana* crown
1. Alkaloids	+	-
2. Saponins	+	+
3. Free Fatty Acids	-	+
4. 2-deoxysugars	+	+
5. Flavonoids	+	+
6. Leucoanthocyanins	+	+
7. Tannins	+ **(hydrolyzable)**	+ **(condensed)**
8. Unsaturated steroid/ triterpenoids	+	+
9. Anthraquinones	-	-
10. Fats and Oils	+	+

Legend: (+) - present, (-) - absent

Conclusion: Plants comprise a vast untapped pool of bioactive phytochemicals that may be widely used in pest control programs instead of the conventional synthetic insectides. The remarkable toxicity effects exhibited by the fruit wastes of *G. mangostana* against the 3rd and 4th instars larvae of *A.aegypti* indicate its potential to provide core structures from which a more sustainable and environmentally-safe larvicidal agents can be synthesized. Further study on the plant should therefore be fully explored. It is worthwhile to study extensively

the larvicidal property by isolating and identifying the active components that cause larval mortality and then use them in field trials in order to assess their full potential as an alternative to chemical larvicides.

Acknowledgements: The National Research Council of the Philippines of the Department of Science and Technology, Taguig City, Metro Manila, Philippines is gratefully acknowledged for providing the research grant and the Industrial Technology Development Institute of the Department of Science and Technology for the unwavering support in the continuous implementation of this project.

REFERENCES

Capeding, M. R., M. N. Chua, S. R. Hadinegoro, I. I. H. M. Hussain, R. Nallusamy, P. Pitisuttithum, K. Rusmil, U. Thisyakorn, S. J. Thomas, N.H. Tran, D. N. Wirawan, I. K. Yoon, A. Bouckenooghe, Y. Hutagalung, T. Laot and T. A. Wartel (2013). Dengue and Other Common Causes of Acute Febrile Illness in Asia: An Active Surveillance Study in Children. PLOS Negl. Trop. Dis., 7(7): 1-9

de Morais, S. M., V. A. Facundo, L. M. Bertini, E. S. B. Cavalcanti, J. F. dos Anjos Júnior, S. A. Ferreira, E. S. de Brito, M. A. de Souza Neto (2007). Chemical composition and larvicidal activity of essential oils from *Piper* species. Biochem. Syst. Ecol., 35 (10): 670–5

Gautam, K., P. Kumar, and S. Poonia (2013).Larvicidal activity and GC-MS analysis of flavonoids of *Vitex negundo* and *Andrographis paniculata* against two vector mosquitoes *Anopheles stephensi* and *Aedes aegypti*. J. Vector Borne Dis., 50 (3):171-8.

Ghosh, A., N. Chowdhury and G. Chandra (2012). Plant extracts as potential mosquito larvicides. Indian J Med Res., 135(5): 581-98

Guevarra, B. Q. (Editor) (2005). A Guidebook to Plant Screening: Phytochemical and Biological. UST Publishing House; Manila (Philippines)

Gutierrez, P. M., A. N. Antepuesto, B. A. L. Eugenio, M. F. L. Santos (2014). Larvicidal Activity of Selected Plant Extracts against the Dengue vector *Aedes aegypti* Mosquito Int. Res. J. Biol. Sci., 3 (4): 23-32

Ji X., A. Bharathi and I. A. Khan (2007). Quantitative and qualitative determination of six xanthones in *Garcinia mangostana* L. by LC–PDA and LC–ESI-MS.J. Pharm. Biomed. Anal., 43(4):1270–76.

Kamaraj, C., A. Abdul Rahuman, A. Mahapatra, A. Bagavan and G. Elango (2010). Insecticidal and larvicidal activities of medicinal plant extracts against mosquitoes. Parasitol.Res., 107 (6):1337–49

Larson, R. T., J. M. Lorch, J. W. Prodgeon, J. J. Becnel. G. G. Clark and Q. Lan (2010).The Biological Activity of α-Mangostin, a Larvicidal Botanic Mosquito Sterol Carrier Protein-2 InhibitorJ Med Entomol., 47(2):249–257

Monzon, R. B, J. P. Alvior, L. L. Luczon, A. S. Morales and F. E. Mutuc (1994). Larvicidal potential of five Philippine plants against *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say).Southeast Asian J Trop Med Public Health, 25 (4):755-9

Pavela, R. (2009). Larvicidal effects of some Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitol. Res., 105 (3):887–92

Pavela, R. (2008). Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae).Parasitol.Res., 102 (3):555–9

Pirali-Kheirabadi, K. and J. A. T. da Silva (2010).*Lavandula angustifolia* essential oil as a novel and promising natural candidate for tick (Rhipicephalus (Boophilus) annulatus) control. Exp. Parasitol., 126 (2):184-6

Shaalan, E. A. S., D. Canyon, M. W. Younes, H. Abdel-Wahaba and A. H. Mansoura (2005). Review of botanical phytochemicals with mosquitocidal potential. Environ Int., 31(8):1149-66

Sutthanont, N., W. Choochote, B. Tuetun, A. Junkum, A. Jitpakdi, U. Chaithong, D. Riyong and B. Pitasawat. (2010). Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and -resistant strains of *Aedes aegypti (*Diptera: Culicidae).J. Vector Ecol., 35 (1) :106-15

Thein, W. M., P. A. Javier, and F. A. Ceballo (2013). Insecticidal Activity of Crude Plant Extracts Against*Sitophilus spp.*Coleoptera: Curculionidae) and *Calloso bruchuschinensis* (L.) (Coleoptera: Bruchidae).Philipp Agric Scientist, 96(2): 154–62.,

Torres, R. C., A. G. Garbo, and R. Z. M. L. Walde (2014). Larvicidal activity of *Persea americana* Mill. Against *Aedes aegypti.* Asian Pac J Trop Med., 7(Suppl 1): S167-S170.

World Health Organization (2005). Instructions for determining the susceptibility or resistance of mosquito larvae to insecticide. WHO/VBC/81.807.