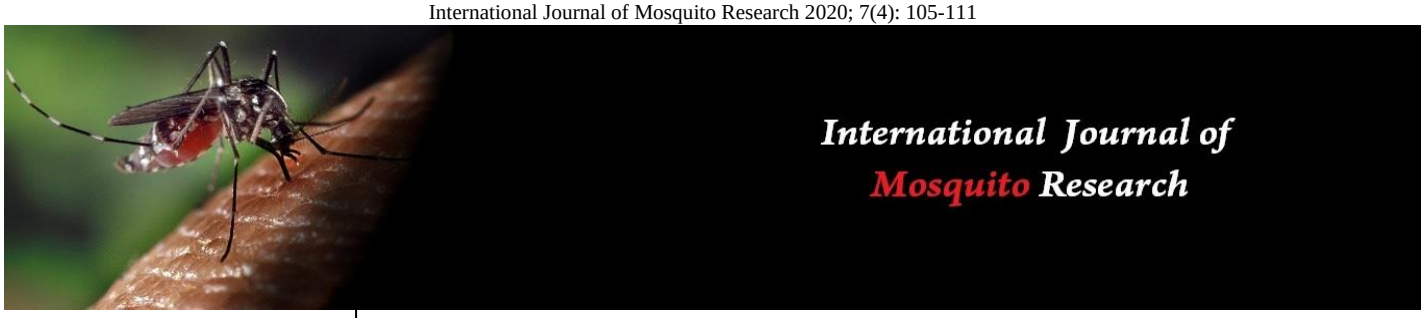


ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2020; 7(4): 105-111

Received: 13-05-2020
Accepted: 15-06-2020

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# Effects of *Lansium domesticum* leaf extract on mortality, morphology, and histopathology of *Aedes aegypti larvae* (Diptera: Culicidae)

**Rizal Subahar, Agus Aulung, Ismalia Husna, Rawina Winita, Lisawati Susanto, Nadar Sukri Lubis and Nurhadi Eko Firmansyah**

**Abstract**
Plant extracts contained bioactive compounds that may kill mosquito larvae. The study aimed to determine an effective concentration of the *Lansium domesticum* leaf extract and its mechanism in causing *Aedes aegypti* larval death. *Ae. aegypti* larvae were exposed to different concentrations of the *L. domesticum* leaf extracts. The lethal concentration 50 ($LC_{50}$) value of the extract was used for fractionation. The histopathological examination of the larval midgut was used H&E stain. At 24 h, $LC_{50}$ and $LC_{90}$ values of the extract were 0.22% and 0.32%, whereas, at 48 h, $LC_{50}$ and $LC_{90}$ values were 0.7% and 1.2% respectively. The larvae became shorter, pale, damage of anal papillae, and the darken siphon. The histopathological larval midgets consisted of the cell protrusion to apical, the detached midgut cells in the lumen, and the irregular cell structure. The *L. domesticum* leaf extract is a potential plant-based insecticide to control *Ae. aegypti* population.

**Keywords:** *Aedes aegypti*, dengue, insecticides, Lansium domesticum

## Introduction
*Aedes* sp. is a vector that can transmit filariasis and a variety of viral diseases such as chikungunya, yellow fever, rift valley fever, Zika virus and dengue hemorrhagic fever (DHF) [1, 2]. *Aedes* species transmitted the dengue virus to humans are *Ae. aegypti* and *Ae. albopictus.* Dengue is a disease caused by 4 strains of Flaviviridae family of viruses, which dengue virus (DENV)-1, DENV-2, DENV-3, and DENV-4. The virus consists of a single-chain RNA is replicated in the body of mosquitoes of the genus *Aedes.* [3]. In 2017, DHF cases were reported in 34 provinces in Indonesia as many as 59,057 and 444 of them died. The data was lower than in 2016 with the number of patients reached 201,885 people and 1,585 of them died [4, 5].
Up till now there is neither drug nor effective vaccine available for dengue treatment and prevention. Elimination of the dengue can be accomplished by vector control, however continuous usage of synthetic insecticides such as carbamate, organophosphate, organochlorine, and pyrethroid insecticides may cause environmental pollution, disruption of ecosystem, non-target organism intoxication, and vector resistance [6]. This urges development of new insecticides that should be effective, environmental friendly and safe to living organisms; development of plant-based insecticide is suitable to this challenge.
The previous studies showed that the seeds and rind of *Lansium domesticum* contain bioactive compounds such as alkaloids, flavonoids, saponins, and polyphenols [7, 8]. Indole alkaloid with structures such as strychnine and quinine which is a compound that functions as an insect repellent [9]. Saponin is known as anti-insect property because of its ability to decrease the activity of the enzyme on digestion and absorption of food. Saponins can also bind free sterols in the digestion of food because sterols act as precursors of ecdysone hormone. The decline in the number of free sterols will disrupt the process of molting in insect [10], and these compounds also interact with larvae cuticle membrane so that it can damage the lining membrane of the larvae [11, 12]. This study aimed to determine an effective concentration of the *L. domesticum* leaf extract, identify the bioactive compounds, and its mechanism in causing the death of *Ae. aegypti* larvae.

## Materials and methods
### Extraction of *L. domesticum* leaves
*L. domesticum* leaves, from Lampung, West Sumatera, 1,000 g (wet weight), were cut into small pieces and dried. Afterward, they were blended into the powder then 300 ml methanol

absolute was added and homogenized, stirred for 24 hours. The solvent was evaporated using a vacuum evaporator [13,14]. A total of 100 g crude extract was recovered.

**Fractionation**
Fractionation was performed using separating funnel with different solvent polarity, i.e. n-hexane, ethyl acetate, and butanol; each of solvents of the obtained fraction was evaporated by vacuum evaporator [15, 16].

**Alkaloid test**
The alkaloid test was performed by thin-layer chromatography (TLC) using silica gel GF254 (CAS.7631-86-9, EC Number: 231-545-4) and corresponding eluent. Eliminated spots were sprayed with Dragendorff's reagents (Sigma-Aldrich, 44578-100MLF). The positive result showed an orange patch on the TLC plate [15, 16].

**Saponin test**
The fractions were dissolved with 10 ml of aquadest and then shaken vigorously. The presence of stable foam for at least 10 minutes, suggests the saponin compound [15, 16].

**Larval Bioassay**
The larval bioassay was conducted under the WHO standard method [17]. Each plastic cup (± 200 ml) contained the crude extract of *L. domesticum* leaves with different concentrations, i.e. 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, and 1.2%. Twenty-five *Ae. aegypti* third and fourth instars larvae were placed into each plastic cup which contained the crude extract and the larval food. The observation was carried out for 24 – 48 h to determine the lethal concentration 50 and 90 ($LC_{50}$ and $LC_{90}$) of each experiment. The bioassays were replicated four times for each concentration.

**Morphological examination**
The morphological examination of the dead larvae was directly performed under the light microscope; the larval size, color, damage of the anal papillae, and the siphon were recorded [18].

**Histopathological examination**
*Ae.aegypti* larvae were fixed and then dehydrated with ethanol and toluene series. Afterward, the larvae were embedded in paraffin section and stained with hematoxylin and eosin (H&E). The result was observed under a compound microscope [18]. The histopathological examination was done at the Department of Anatomic Pathology, Faculty of Veterinary Medicine, IPB, Bogor, West Java, Indonesia.

**Data analysis**
Data were expressed as descriptive statistics and analyzed by statistical package for social sciences (SPSS) ver.24. Data on the mortality rate of the dead larvae were tested for normal distribution (Shapiro-Wilk). Data with normal distribution were analyzed by analysis of variance (ANOVA), while the non-normal one by Kruskal-Wallis. $LC_{50}$ and $LC_{90}$ values were performed by Probit analysis with a 95% confidence interval, $p < 0.05$ was considered statistically significant.

**Results**
At 24 h exposure, 56–72% dead larvae were found at 0.1–0.2% concentrations (Figure 1), while at 48 h, 100% dead larvae were found at minimum concentration of 0.2% (Figure 2). The larval mortality was higher in accordance with the longer exposure time. At 24 h exposure, $LC_{50}$ and $LC_{90}$ values were 0.22% (95% CI=0.18–0.25) and 0.32% (95% CI=0.27–0.42), while at 48 h, $LC_{50}$ and $LC_{90}$ values were at 0.7% (95% CI=0.00–0.16) and 1.2% (95% CI=0.00–0.23) respectively (Figure 3).
At 24 h, mean (SD) of the dead larvae as follows; 0.1% concentration of the extract showed mean (SD) of the dead larvae was 14 (2.9), 0.2% 18 (2.9), 0.4% 25 (0.0), 0.6% 25 (0.0), 0.8% 25 (0.0), 1.0% 25 (0.0), 1.2% 25 (0.0). At 48 h, 0.1% concentration of the extract showed mean (SD) of the dead larvae 19 (1.3) and 0.2–1.2% showed mean (SD) of the dead larvae 25 (0.0), respectively. Statistical results, Kruskal-Wallis test, showed that there were differentiation between concentration of the extract and mean (SD) of the dead larvae ($p = 0.000$, $p < 0.05$).

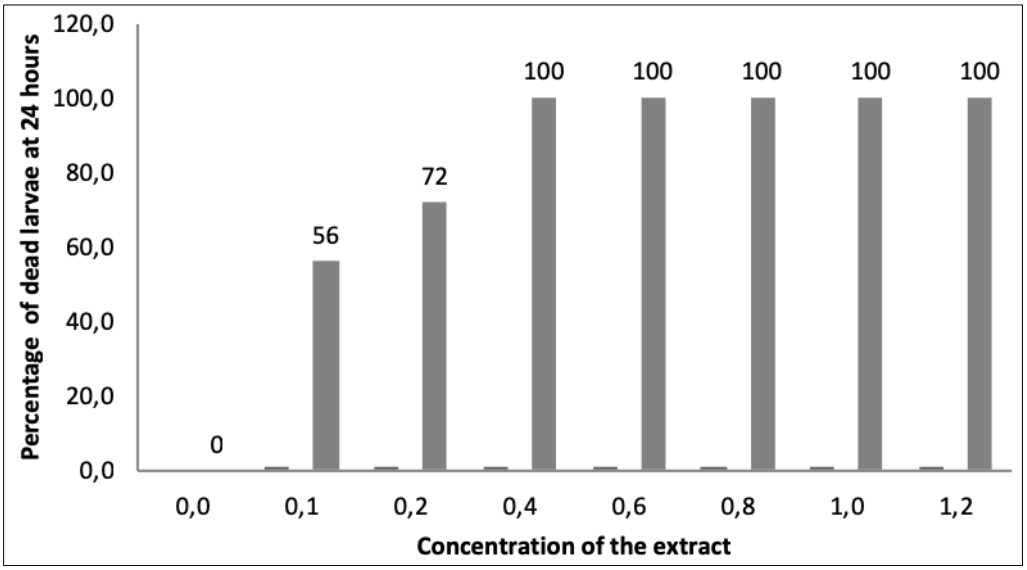



**Fig 1:** Percentage of dead larvae at 24 hours

Figure 1 shows that the percentage of dead larvae at 24 hours. The concentration of the extract, 0.1–0.2% have 56–72% dead larvae. In contrast, 0.4–1.2% concentration of the extract exhibited 100% dead larvae respectively.

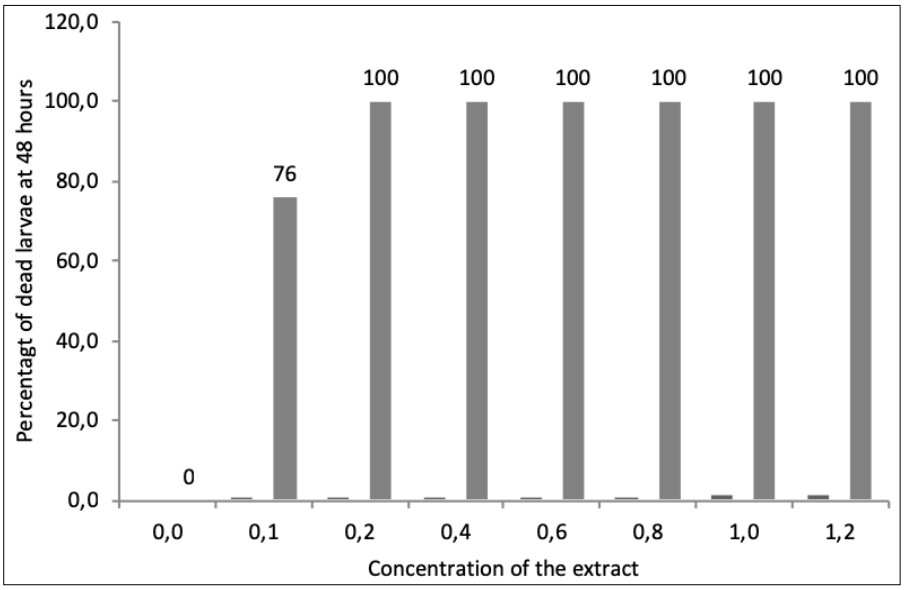



**Fig 2:** Percentage of dead larvae at 48 hours

Figure 2 shows that the percentage of the dead larvae at 48 h. Only the concentration of the extract, 0.1% showed 72% dead larvae. In contrast, 0.2 – 1.2% concentration of the extract exhibited 100% dead larvae respectively.

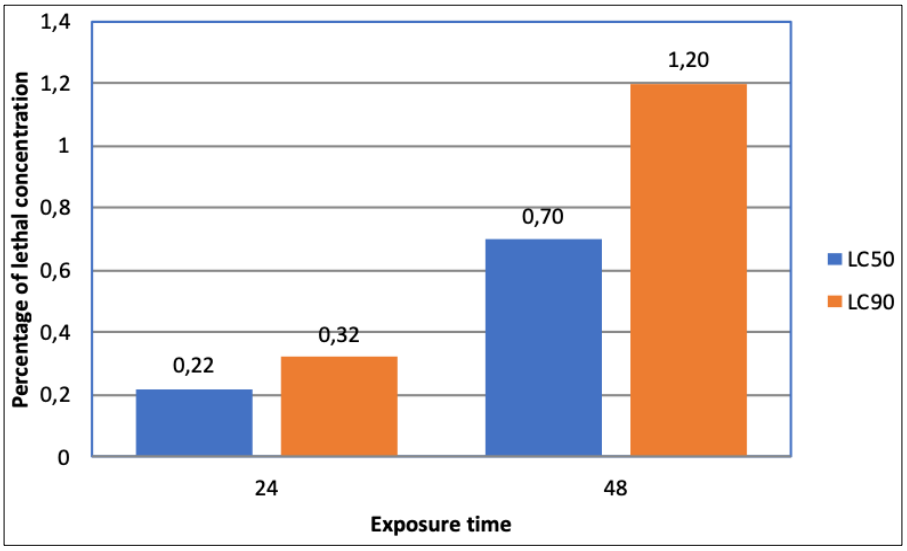



**Fig 3:** Lethal concentration (LC) after 24 and 48 hours

*Ae. aegypti* larvae of the control group have an average length of 4 – 5 mm and the color was transparent. In contrast, the control group, morphological changes were observed in the treatment group i.e. size decrease, body surface damaged, the color became dark or pale, damage of the anal papillae, and darken of the siphon (Figure 4).

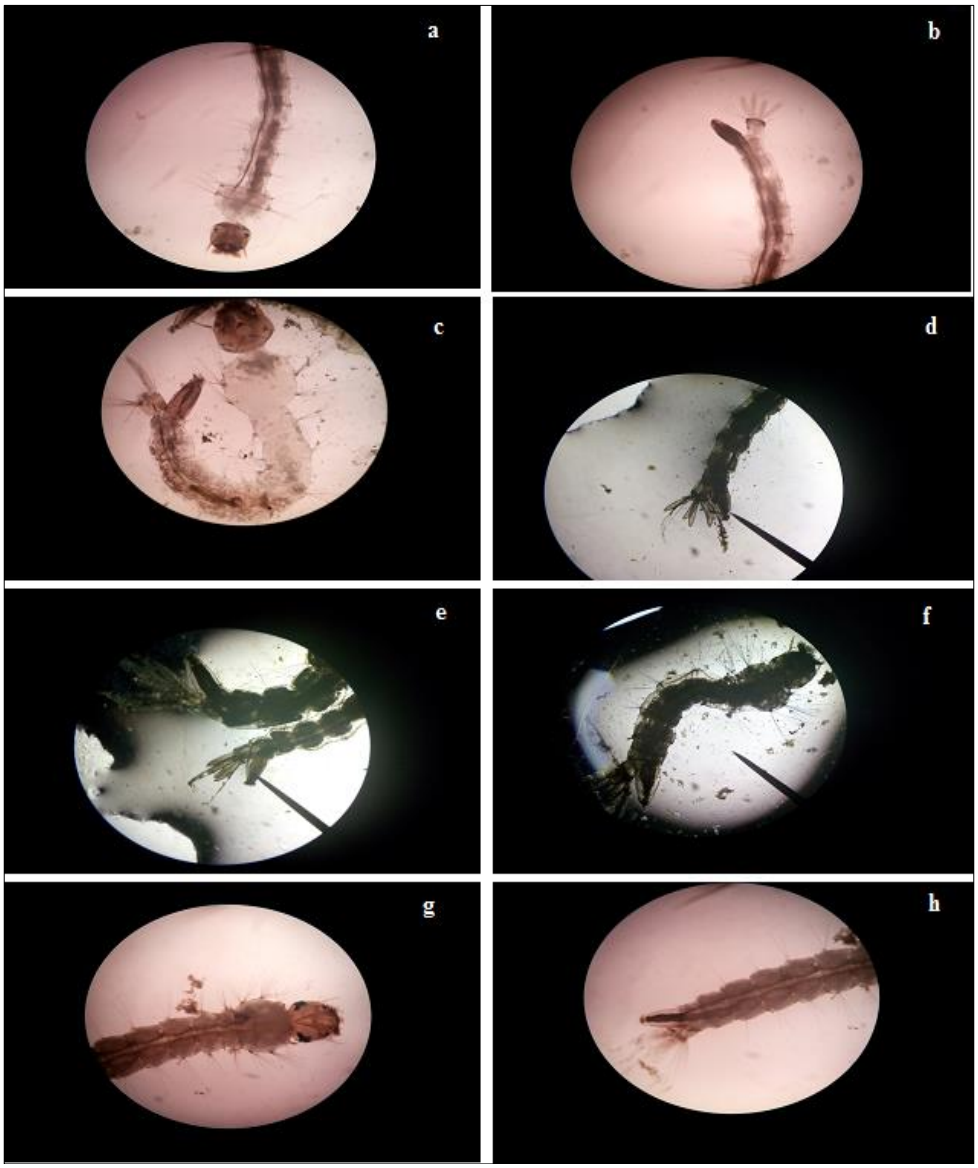
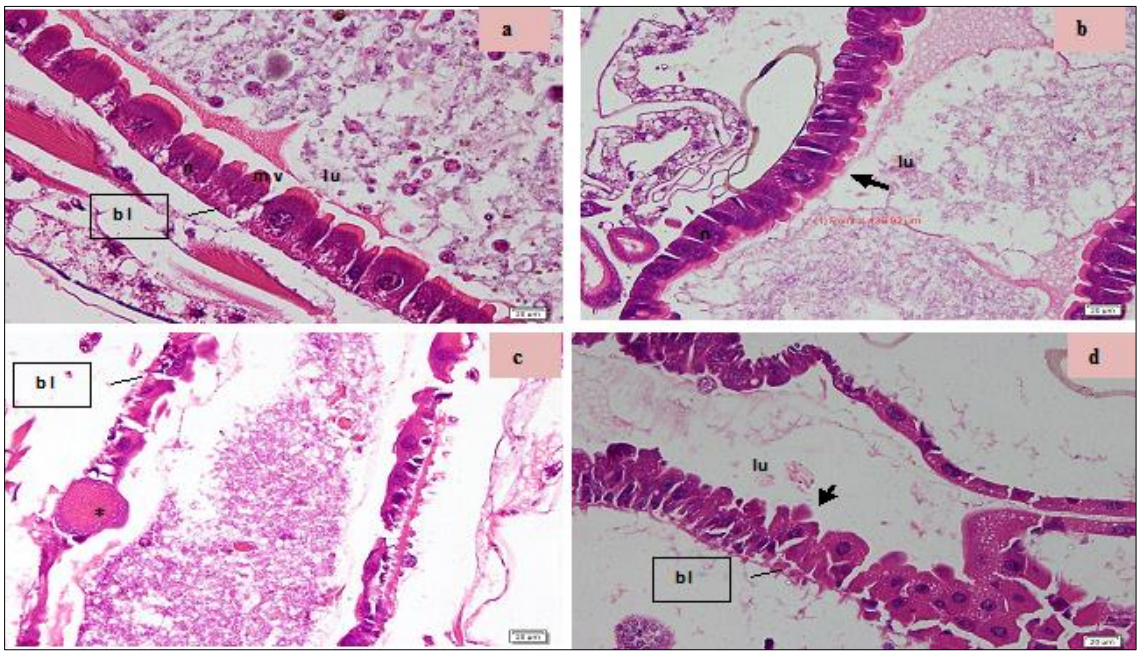



**Fig 4:** Effect of crude extract and fraction of *L. domesticum* leaves towards morphology change of *Ae. aegypti* larvae. (a) and (b) control; (c) crude extract; (d) ethyl acetat fraction; (e) dan (f) hexane fraction; (g) dan (h) butanol fraction (magnification 40x)



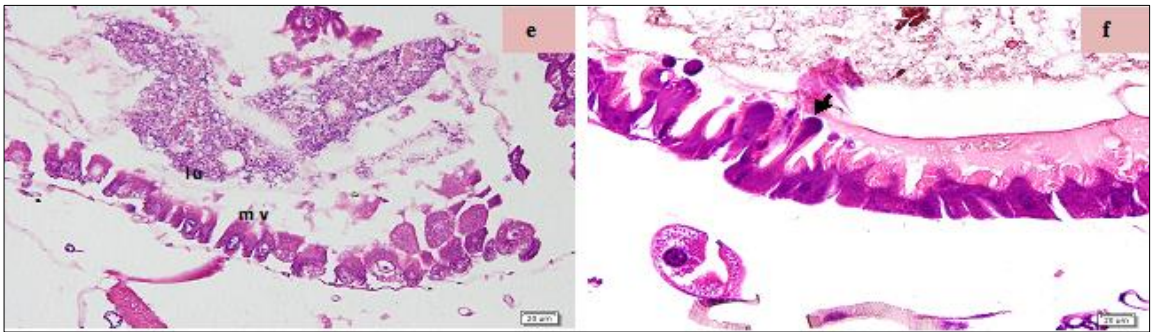



**Fig 5:** Histopathology of midgut *Ae. aegypti* larvae from control group (a) and treatment (b–f). (b and c) crude extract; (d) ethyl acetat fraction; (e) hexane fraction; and (f) butanol fraction. All of the midgut showed destructive cells n=nucleus; mv=microvilli; bl=basal lumina; lu=lumen; (*)=swelling cell; black arrow=protrude cell (magnification 200x).

Figure 5 shows the histopathology section of the larva midgut stained with HE. In the control group showed a good structure of the epithelial cells, luminal, microvilli, and basal lamina (Figure 5a). In contrast, in the treatment group the midgut of *Ae. aegypti* larvae showed the apical protrusion in the epithelial cells and swelling (Figures 5b and c) or the irregular cell structures (Figure 5d), or the cells were shrinkages and the microvilli was broken (Figure 5e), and elongation of the midgut cells (Figure 5e).

## Discussion

This study showed the potential of the *L. domesticum* extract to control *Ae. aegypti* larvae with the $LC_{50}$ value of 0.22% and $LC_{90}$ of 0.32% for 24 h. *L. domesticum* is one of the medicinal plants from family Meliaceae like *Dysoxylum malabricum*, *Azadirachta indica*, *Melia volkensii*, *Melia azedarach*, *Khaya senegalensis*, *Turraea abyssinica* [19]. The extraction of the *L. domesticum* leaves used the methanol as a solvent with the polarity index of 5.1. The methanol was believed to dissolve almost all of the secondary metabolites from the extracts [20]. Suryanto and Wehantouw [21] reported that the methanol is more superior than the ethanol in dissolving the secondary metabolite of *Artocarpus altilis* F. It was suggested that the methanol is recommended to be used as a solvent when the study of medicinal plants focused on the larvicidal and adulticidal activity.

Alkaloids and saponins are the main bioactive compounds of the *L. domesticum* leaves. These phytochemicals play a role as an insect repellent by inhibiting acetylcholinesterase. Acetylcholine acts as a sodium channel and transmits impulses to the larval nervous system. When acetylcholinesterase is inhibited, acetylcholine cannot diffuse into the membrane postsynapse to bind receptors. As a result, it causes the damage of the larval muscles and nerves; the larvae become be spastic and eventually die [22].

Alkaloids could also act as stomach poison, disrupting the digestive system, and inducing *Ae. aegypti* larval death [23]. The larvae's food like pellet was added with the extract and fraction of *L. domesticum*, they will enter the larvae cell and inhibit cell metabolism processes in mitochondria, causing lack of energy. The present study was in line with Liu *et al*. [24] reported that alkaloids can be toxic for the larvae which taken the food from the water surface. Moreover, alkaloids inhibit the third larval growth hormones, namely the brain, edonone, and growth hormones. When these hormones do not work properly, the larvae will fail to develop into the pupae and eventually die.

A saponin is well known as an insecticidal property, because it decreases activity of the digestive enzymes and food absorption. The saponin can also bind to free sterols in the insect digestion. Free sterols act as a precursor of the ecdysone hormone. When free sterols are a low concentration, they inhibit a molting process. Moreover, the saponin has an antifeedant activity and inhibits the larval growth and its interactions with the cuticle membrane. Finally, they cause damage of the larvae and die [25].

Morphology alterations of the larvae in the treatment group became shorter in size than the control, suggesting the damage of the larval exoskeleton (chitin) [23]. Chitin functions as body protection and limits the water loss through body walls. The larval body wall absorbs toxic substances easily [26]. The color of the extract-exposed larvae became pale, white, even darken in comparison to the control larvae which were transparent, referring to the dead or dying tissues [23]. The larval siphon which is a main component of the larval respiratory system became pale and/or dark in color. The damage of the siphon will remove the larval hydrophobic surface and subsequently, water cannot enter into the larval body and the larvae lacked the oxygen. Therefore, the damage of the anal papillae and siphon contributed the larval viability [27].

The larval midgut has an important role in secret digestive enzymes and to absorb nutrients [24]. The damage that occurred in the larval midgut in this study consisted of protrusion of the epithelial cells and swollen cells in the treatment groups. The damages will influence the digestion process in the larval midgut as the crude extract of *L.domesticum* could act as a contact poison. The mechanism can be explained as follow: after several hours of exposure, the toxins in the crude extract and the fraction passing through the cell membrane and bound to special receptors on the epithelial cells to form the small pores. They lead to the cell osmotic balance impaired and the ions and water easily enter the cells, resulting in the cells to expand then lysed. The lysed cells became separated from the basal membrane and released into the midgut lumen [28].

The potential of the *L. domesticum* extract has been studied in the medical application. For example, Rohin *et al*. [29] showed that a proliferative effect of the Duku (*L. domesticum Corr*) extracts on the human colorectal adenocarcinoma cell lines. Monosroi *et al*. [30] demonstrated that the fruit extract of *L. domesticum* contained anticancer active compounds such as phenolic substances and plavonoid against KB cell (human mouth epidermal carcinoma cell) because of its high anti-proliferative and MMP-2 (matrix metalloproteinase-2) inhibition activities. The seed extract of *L. domesticum*

contained tetranortriterpenoids showed the antimalaria activity against *Plasmodium falciparum* [31]. Therefore, the extract of *L. domesticum* contained bioactive compounds may be used in the clinical aspects.

In conclusion, the *L. domesticum* leaf extract caused the mortality of *Ae. aegypti* larvae with an $LC_{50}$ value of 0.22% and $LC_{90}$ of 0.32% for 24 h exposure. Morphological alterations of *Ae. aegypti* larvae consisted of shorter in size, pale, white, and dark in color. The *L. domesticum* leaf extract caused the histopathological midgut alterations of the *Ae. aegypti* larvae consisted of the apical protrusion in the epithelial cells and swelling or the irregular cell structures or the cells were shrinkages and the microvilli were broken, and elongation of the midgut cells.

## Conflict Interest

Authors declare no conflict interest

## Acknowledgement

The authors thank to the Head of Department of Parasitology, Universitas Indonesia; Biochemistry and Molecular Biology Laboratory, Universitas Indonesia; Chemistry Laboratory, Universitas Indonesia; Laboratory of Pharmacy, Universitas Pancasila; Histology Laboratory, Institut Pertanian Bogor; and the Laboratory of Botany, Universitas Lampung, for giving permission to conduct this research.

## Funding Sources

This study was funded by personal funds from Ismalia Husna, and by Directorate Research and Community Engagement (DRPM), University of Indonesia, Indonesia, Project of Hibah PITTA 2018. We are grateful to thank the Department of Parasitology, Faculty of Medicine, University of Indonesia, Indonesia for their technical support. Furthermore, we would like to express our deepest gratitude and especial respect to all participants in this study.

## References

1. Souza-Neto JA, Powell JR, Bonizzoni M. *Aedes aegypti* vector competence studies: a review. Infection, Genetics and Evolution. 2019; 67:191-209.
2. Wong PSJ, Li MZI, Chong CS, Ng LC, Tan CH. *Aedes* (*Stegomyia*) *albopictus* (Skuse): a potential vector of zika virus in Singapore. PLoS Negleted Tropical Diseases. 2013; 7(8):e2348.
3. Guerdan BR. Dengue fever/dengue hemorrhagic fever. American Journal of Clinical Medicine. 2010; 7(2):51-3.
4. The Ministry of Health of Republic of Indonesia. Data and profile information on Indonesian health. Ministry of Health of the Republic of Indonesia, 2018, 154.
5. The Ministry of Health of Republic of Indonesia. Data and profile information on Indonesian health. Ministry of Health Republic of Indonesia, 2017, 137.
6. Kumar S, Wahab N, Mishra M, Warikoo R. Evaluation of 15 local plant species as larvicidal agents against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). Frontier in Physiology. 2012; 3:104.
7. Klungsupya P, Suthepakul N, Muangman T, Rerk-Am U, Thongdon-AJ. Determination of free radical scavenging, antioxidative DNA damage activities and phytochemical components of active fractions from *Lansium domesticum* Corr. Fruit. Nutrients. 2015; 7(8):6852-73.
8. Munir T, Munawar KS, Mohyuddin A. An overview of the antibacterial implication of *Lansium domesticum*. Journal of Basic & Applied Sciences. 2018; 14:206-9.
9. Miettinen K, Dong L, Navrot N, Schneider T, Burlat V, Pollier J *et al*. The seco-iridoid pathways from *Catharanthus roseus*. Nature Communications. 2014; 5:3606.
10. Chaieb I. Saponin as insecticides: a review. Tunisian Journal of Plant Protection. 2010; 5(1):39-50.
11. Deore SL, Khadabadi SS. Larvicidal activity of the saponin fractions of *Chlorophytum borivilianum Santapau* and *Fernandes*. Journal of Entomology and Nematology. 2009; 1(5):064-6.
12. Elumalai K, Dhanasekaran S, Krishnappa K. Larvicidal activity of saponin isolated from *Gymnema sylvestre* R. Br. (Asclepiadaceae) against Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). European Review Medicine and Pharmacology Science. 2013; 17(10):1404-10.
13. Marliana SD, Suryanti V, Suyono. The phytochemical screenings and thin layer chromatography analysis of chemical compounds in ethanol extract of labu siam fruit (*Sechium edule* Jacq. Swartz.). Biofarmasi. 2005; 3(1):26-31.
14. Harborne AJ. Phytochemical methods: A guide to modern technique of plant analysis. Third Ed. Springer Netherland. 1998; XIV:302.
15. Mukhtar MU, Mushtaq S, Arslan A, Zaki AB, Khaleeq H, Bhatti A. Laboratory study on larvicidal activity of different plant extracts against *Aedes aegypti*. International Journal of Mosquito Research. 2015; 2(3):127-130.
16. WHO. Guidelines for laboratory and field testing of mosquito larvicides. WHO, Geneva, 2015.
17. Alves SN, Serrão JE, Melo AL. Alteration in the fat body and midgut of *Culex quinquefasciatus* larvae following exposure to different insecticides. Micron. 2010; 41(6):592-7.
18. Al-Mehmadi RM, Al-Khalaf AA. Larvicidal and histological effects of *Melia azedarach* extract on *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Journal of King Saud University-Science. 2010; 22(2):77-85.
19. Baskar K, Mohankumar S, Sudha V, Maheswaran R, Vijayalakshmi S, Jayakumar M. Meliaceae plant extracts as potential mosquitocides-a review. Entomology, Ornithology & Herpetology. 2016; 5(1):172.
20. Widyawati PS, Budianta TD, Kusuma FA, Wijaya EL. Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia Less* leaves extracts. International Journal of Pharmacological and Phytochemical Reseach. 2014; 6(4):850-5.
21. Suryanto E, Wehantouw F. Free radical scavenging activities of phenolic extracts from bread fruit leaves. Progress Chemistry. 2009; 2(1):1-7 (Indonesian).
22. Deuis JR, Mueller A, Israel MR, Vetter I. The pharmacology of voltage-gated sodium channel activators. Neuropharmacology. 2017; 127:87-108.
23. Kim SI, Ahn YJ. Larvicidal activity of lignans and alkaloid identified in *Zanthoxylum piperitum* bark toward insecticide-susceptible and wild *Culex pipiens pallens* and *Aedes aegypti*. Parasites & Vectors. 2017; 10:221.
24. Liu ZL, Liu QZ, Du SS, Deng ZW. Mosquito larvicidal activity of alkaloids and limonoids derived from *Evodia rutaecarpa* unripe fruits against *Aedes albopictus*

(Diptera: Cillicidae). Parasitology Research. 2012; 111(3):991-6.
25. Podolak I, Galanty A, Sobolewska D. Saponin as cytotoxic agents: a review. Phytochemical Review. 2010; 9(3):425-4.
26. Mezendorfer H, Zimoch L. Chitin metabolism in insects: structure, function, and regulation of chitin synthases and chitinases. Journal of Experimental Biology. 2003; 206:4393-412.
27. Yu KX, Wong CL, Ahmad R, Jantan I. Larvicidal activity, inhibition effect on development, histopathological alteration and morphological aberration induced by seaweed extracts in *Aedes aegypti* (Diptera: Culicidae). Asian Pacific Journal of Tropical Medicine. 2015; 8(12):1006-12.
28. Procópio TF, Fernandes KM, Pontual EV, Ximenes RM, de Oliveira AR, Souza Cde S *et al*. *Schinus terebinthifolius* leaf extract causes midgut damage, interfering with survival and development of *Aedes aegypti* larvae. PLoS ONE. 2015; 10(5):e0126612.
29. Rohin MA, Jumli MN, Ridzwan N, Hadi NA, Ismail S, Hussin TM *et al*. Anti-proliferative effect of duku (*Lansium Domesticum Corr*) extracts on human colorectal adenocarcinoma cell lines. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016; 7(2):1078-83.
30. Manosroi A, Chankhampan C, Manosroi W, Manosroi J. Anti-proliferative and matrix metalloproteinase-2 inhibition of Longkong (*Lansium domesticum*) extracts on human mouth epidermal carcinoma. Pharmaceutical Biology. 2013; 51(10):1311-20.
31. Saewan N, Sutherland JD, Chantrapromma K. Antimalarial tetranortriterpenoids from the seeds of *Lansium domesticum* Corr. Phytochemistry. 2006; 67(20):2288-93.