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Larvicidal Activity of Ethyl Acetate Leaf Extract of *Aegle marmelos* (L.) Correa Against *Aedes aegypti*

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ABSTRACT

Aegle marmelos **leaf contains secondary metabolites that have bioinsecticidal effects. This study aimed to analyze phytoconstituents of the ethyl acetate extract of *Aegle marmelos* leaves, its larvicidal activity, and its effects on histopathological changes of the midgut of *Aedes aegypti* larvae. The ethyl acetate extract was obtained from the concentrated maceration of the leaf powder and analyzed with GCMS. The instar III or IV larvae were used and divided into six groups where each group was exposed to an extract with a concentration of 1%; 4%; 8% and 16%, and one other control group without exposure to such extract. The histopathology of larval midgut was prepared, stained with Hematoxyllin-Eosin, and observed using light microscopy. GC-MS analysis revealed that the extracts contain 8 compounds, Oleic acid, 9-Hexadecenoic acid, Cis-1,3-Eicosenoic acid, Dasycarpidan-1-methanol, acetate (ester), Digitoxin, Z-(13,14-epoxy)tetradeg-11-en-ol acetate; 2,3-dimethyl-5-trifluoromethyl-1-phen-1,4-diol, ethyl iso-allocholate. Probit test revealed that the LC_{50} value of ethyl acetate extract of *Aegle marmelos* leaves was 3.917% and histopathological results are damage to brush borders, nucleus and nucleolus, epithelial cells, and hypertrophy of the gastric caeca. The leaf ethyl acetate extract from *Aegle marmerlos* can be considered as a probable natural insecticide to kill Aedes aegypti. *Aegle marmelos* can be applied as natural insecticide to eradicate the population of *Ae. aegypti*.**

1. Introduction

Mosquitoes are small insects that are abundant in nature. *Ae. aegypti* belongs to a mosquito that has stripes on its legs. *Ae. aegypti* may cause many diseases, such as chikungunya, dengue fever, filariasis, yellow fever, Japanese encephalitis (Sarma *et al.* 2017). Several studies reported the resistance of *Ae. aegypti* to several household insecticides such as organochlorines, organophosphates, and carbamates. Additionally, the use of these insecticides result in very detrimental impacts on the environment and human health. Therefore, alternative and natural or biodegradable insecticides are needed (Reegan *et al.* 2015). One of the alternatives is using leaf extract of *Aegle marmelos* (local name: maja, Rutaceae).

Our previous research showed that the leave ethyl acetate extract (LEAE) has stronger larvicide activity than leave ethanolic extract of *Aegle marmelos* (Sari and Susilowati 2019). In this study, further investigation was carried out to evaluate the effectiveness of the leave ethyl acetate extract (LEAE). The investigation was covering three aspects, namely identification of the phytoconstituentsin LEAE, *Ae. aegypti*-larvicidal activity, and the histopathological change in the larvae midgut. With these aspects, a decision can be made whether LEAE from *Aegle marmelos* is a good natural insecticide to eradicate *Ae. aegypti*. The effectiveness of LEAE from *Aegle marmelos* (local name: maja, Rutaceae) in eradicating *Ae. aegypti* depends on its bioactive components (Patel *et al.* 2012). In this study, the phytoconstituents of the LEAE were analysed with GC-MS and then associated with the reported bioactivities, particularly in their larvicidal capacity. So far, no report is available on the phytochemical constituents of the LEAE.

Many previous studies on larvicidal capacity of *Aegle marmelos* used Cx. quinquefasciatus larvae.

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Various extracts (petroleum ether, chloroform and ethanol) from *Aegle. marmelos* leave caused 100% mortality in Cx. quinquefasciatus larvae Manimegalai and Annapoorani (2013). Essential oils of *Aegle. marmelos* were found to have ovicidal and repellent agents against *Ae. aegypti* (Sarma *et al.*2017). Leave aqueous extract from *Aegle. marmelos* containing nanoparticles was proved to *Ae. aegypti* (Angajala *et al.* 2014). So, study on the larvicidal activity of LEAE from *Aegle. marmelos* is not yet done.

Effectiveness of the larvicidal capacity of any plant extracts can be traced from their effect on the larvae midgut epithelium cells. Al-Mehmadi and Al-Khalaf (2010) reported that *Melia azedarach* extract that has effective larvicidal activity and can cause damage to midgut epithelial cells due to cytoplasmic vacuolization, damages in microvilli and finally the cell death. Changes in midgut of mosquito larvae will influence the function of the digestive processes such as absorption, ion transport, synthesis of digestive enzymes and osmoregulation processes. Phytochemical compounds found in extract can cause cellular dysfunction or death of the epithelial cells. Again, this study was the first report on the effect of LEAE from *Aegle. marmelos*.

Based on the above three aspects, this study was carried out with objectives to identify and to evaluate the presence of phytoconstituents of LEAE from *Aegle. marmelos* to determine the *Ae. aegypti*-larvicidal activity, and to investigate the effect of the LEAE to the histopathological changes of the larvae midgut epithelium.

2. Materials and Methods

2.1. Herbal Material and its Extraction

The leave of *Aegle. marmelos* (Figure 1) were obtained from the tree that grew in the campus estate of this university (Krida Wacana Christian University, Jakarta). One of the authors, RPS, identified the tree as *Aegle. marmelos* based on the determination key in the Flora of Java. The leave were dried indoors at room temperature. Leave powder (100 g) was macerated with ethyl acetate (500 ml). The macerates were first filtered through Whatman No.1 filter paper, and then evaporated using a rotary evaporator (Rotavor R-3, Buchi) to obtain the leave ethyl acetate extract (LEAE) for further analysis.

2.2. GC-MS Analysis

The GC-MS instrument used was GC Agilent 7890B and MS Agilent 7000 (USA). The column was Agilent HP-1MS (30 m × 0.25 mm, 0.25 µm). The carrying gas was helium with pressure at 1.987 psi, flow: 0.43762 ml/min, velocity: 24.671 cm/sec, total flow: 17.879 mL/min. The sample volume injected was 1 µL into GC-MS. The column oven temperature was 100°C. The injection was 310°C. The septum purge flow was 3 ml/min. The injection mode was split. The split ratio was 33:1. The split flow was 14.441 ml/min.

Figure 1. Leave of *A. marmelos*

Equilibration time was 3 min. The agilent libraries for GC-MS were NIST and Wiley libraries.

2.3. *Aedes aegypti*-larvicidal Activity Test

Ae. aegypti eggs were obtained from the Settlement Pest Control Study Unit, Bogor Agricultural University and then developed in the research laboratory of Faculty of Medicine and Health, Christian Krida Wacana University to become stage III/IV larvae. The ethyl acetate extracts (LEAE) are in four concentrations, as 1,000; 4,000; 8,000 and 16,000 ppm. The positive control is Temephos 1% (1,000 ppm). The negative control is distilled water. Replications for all treatments were four times. The length of the experiment was 24 h. The LC_{50} and LC_{90} were calculated using Probit analysis.

2.4. Histopathological Studies

Instar III/IV larvae *Ae. aegypti* from the treatments (4,000 and 16,000 ppm) were preserved in ethanol 70%. The histopathological preparations were made in the Histology Laboratory of the Faculty of Medicine, Airlangga University, Surabaya. After dehydration of the larvae in a serial graded ethanol, the dehydrated larvae were immersed in each ethanol serial solution for 15 min. All samples were embedded in Historesin JB4. The resulting blocks were sliced by a microtome to obtain a series of 3 µm thick sections. These sections were stained with hematoxylin-eosin and then observed by a light microscope (Olympus CH_20). The histological changes in larvae midgut were documented and compared descriptively.

2.5. RT-LAMP Results Analysis

The data from the larvicidal tests (LC_{50} and LC_{90}) were analyzed with Probit analysis using SPSS (Chi-Square analysis). The influence of extract concentration was confirmed with one-way ANOVA and least significance difference (LSD).

3. Results

3.1. Phytoconstituents of LEAE

GC-MS analysis revealed that the extracts contain eight compounds (Table 1). Three unsaturated fatty acids were identified in LEAE from Aegle. marmelos. They were Oleic acid, 9-Hexadecenoic acid, and cis-1,3-Eicosenoic acid (Figure 2-4). The other five compounds were Dasycarpidan-1-methanol, acetate (ester), Digitoxin, Z-(13,14-epoxy)tetradeg-11-en-ol acetate; 2,3-dimethyl-5-trifluoromethyl-1-phen-1,4-diol, ethyl iso-allocholate (Figure 3). The reported bioactivities of these bioactive compounds are described in Table 2. Many of them have anti larvicidal activities.

3.2. *Aedes aegypti*-larvicidal Activity

The Probit analysis of the larvicidal activity data showed that the effect of LEAE of *Aegle. marmelos* was dependent on the concentration of the extract (Figure 4). All the concentrations above 1% extract, have incurred mortalities proportional to the level of concentration. No mortality was observed at 1,000 ppm of the extract. The insecticide or larvicidal effect of the extract was observed from 4% with 75% mortality. Then, the 83% mortality was observed at 8,000 ppm. The 100% mortality was observed at 16,000 ppm. The negative control was 0% mortality. The positive control (Termephos 1%) was 100% mortality (Figure 5). Probit test revealed that the LC_{50} value of ethyl acetate extract of *Aegle. marmelos* leaves was 3,917 ppm which means that it is estimated that a concentration of 3,917 ppm *Aegle. marmelos* leaves can kill 50% of the exposed populations of *Ae. aegypti* larvae. The LC_{90} was 7,341 ppm. It is recommended that the leave ethyl acetate extract of *Aegle. marmelos* with a concentration of 16.000 ppm is the highest mortality and equivalent to a positive control (Table 3).

Table 1. Bioactive compounds in the extract as analyzed by GC-MS

Compound	Formula	Retention time	M/Z	Relative abundance (%)
Oleic acid	$C_{18}H_{34}0_2$	14.860	69;73;81	19.34
9-Hexadecenoic acid	$C_{16}H3_00_2$	15,589	69;73;81	19.34
Cis-13-eicosenoic acid	$C_{20}H_{38}0_2$	19,728	73;69;55	5.54
Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_20_2$	15.589	69;73;81	9.47
Digitoxin	$C_{41}H_{64}0_{13}$	17.839	73; 57; 69	25.40
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}0_3$	19.529	69; 57; 95	9.17
2,3-dimethyl-5-trifluoromethyl-phen-1,4-diol	$C_9H_9F_30_2$	20.443	73; 69; 55	12.00
Ethyl iso-allocholate	$C_{26}H_{44}0_5$	24.666	129; 84; 57	12.60

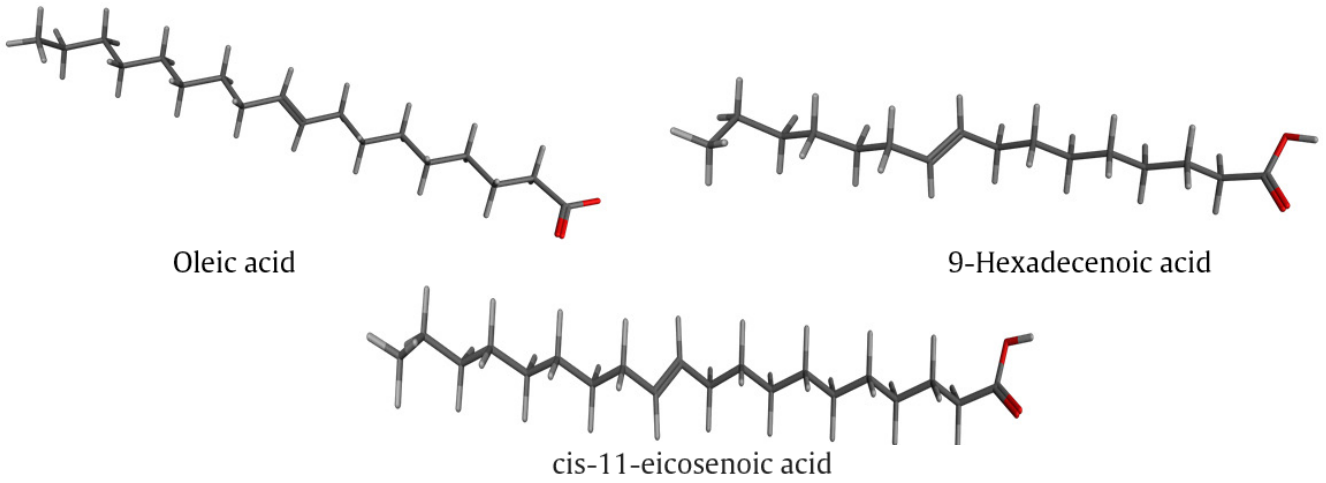

Figure 2. Structure 3D from software moleculer procedure environment (MOE)

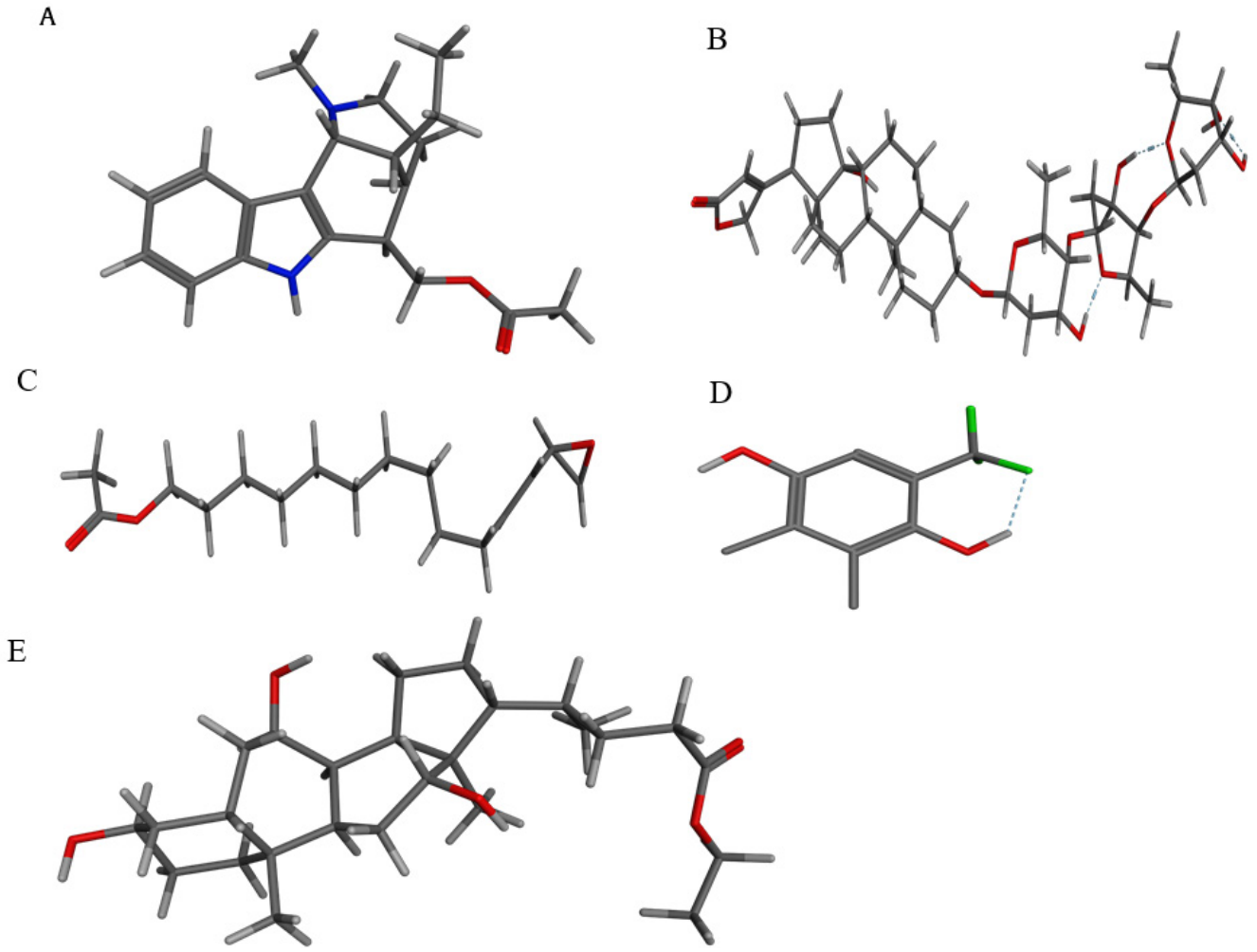

Figure 3. Structure 3D from software moleculer procedure environment (MOE). (A) Dasycarpidan-1-methanol acetate (ester), (B) digitoxin, (C) Z-(13,14-epoxy) tetradec-11-en-1-ol-acetate, (D) 2,3-dimethyl-5-trifluoromethyl-phen-1,4-diol, (E) ethyl iso-allocholate

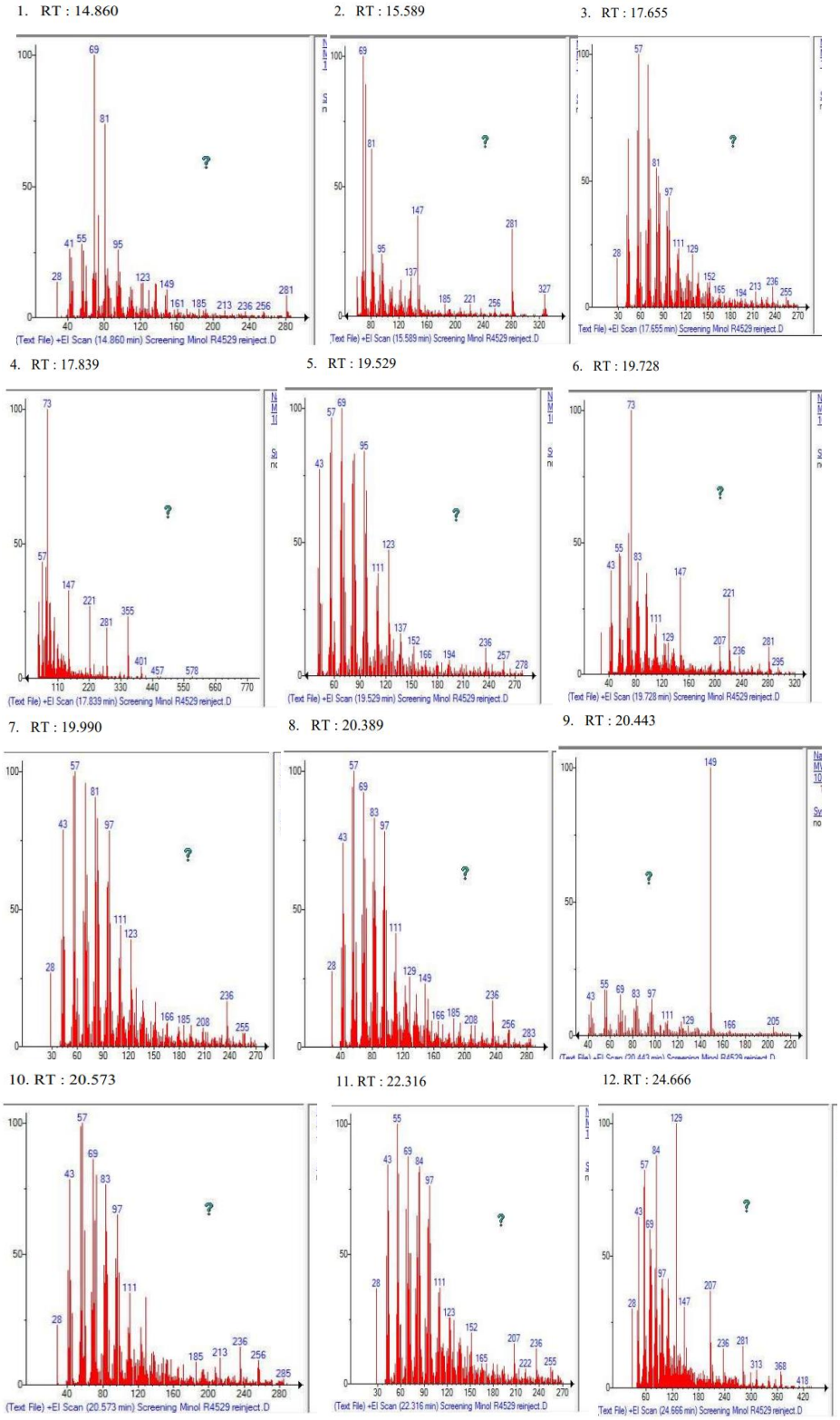

Figure 4. Mass spectra of the identified compounds (see Table 1, for the name of the compounds according to their RT)

Table 2. Bioactivity of compounds in the leave ethyl acetate extract from *Aegle marmelos*

Bioactive Compound	Bioactivity	Bioactivity
Oleic acid	Larvicidal Antifeedant and pupicidal	Cantrell *et al.* (2011), Gurunathan *et al.* (2016), Perumalsamy *et al.* (2015), Rahuman *et al.* (2008), Kannathasan *et al.* (2008), (Kamaraj *et al.* (2021)
9-Hexadecenoic acid	Larvicidal, insecticidal Antifeedant and pupicidal	Farag *et al.* (2021), Kamaraj *et al.* (2021)
Cis-13-eicosenoic acid	Larvicidal, antimicrobial	Karthi *et al.* (2020)
Dasycarpidan-1-methanol, acetate (ester)	Anticancer, antimicrobial	Moni *et al.* (2021)
Digitoxin	Antitumor Cardioactive steroids Antileishmanial	Eldawud *et al.* (2020), Ershad *et al.* (2020) Freitas et al (2021)
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	No information available	
2,3-dimethyl-5-trifluoromethyl-phen-1,4-diol	Anti-inflammatory	Abdulhafiz *et al.* (2020)
Ethyl iso-allocholate	Antioxidant Antibebesial potential Anti-inflammatory Anticancer Antimicrobial Antivirus	Boligon *et al.* (2013), Prakash *et al.* (2019), Guz *et al.* (2021), Johnson *et al.* (2020), Okoye *et al.* (2011), Shah *et al.* (2021), Thakur and Ahirwar (2019), Poochi *et al.* (2020)

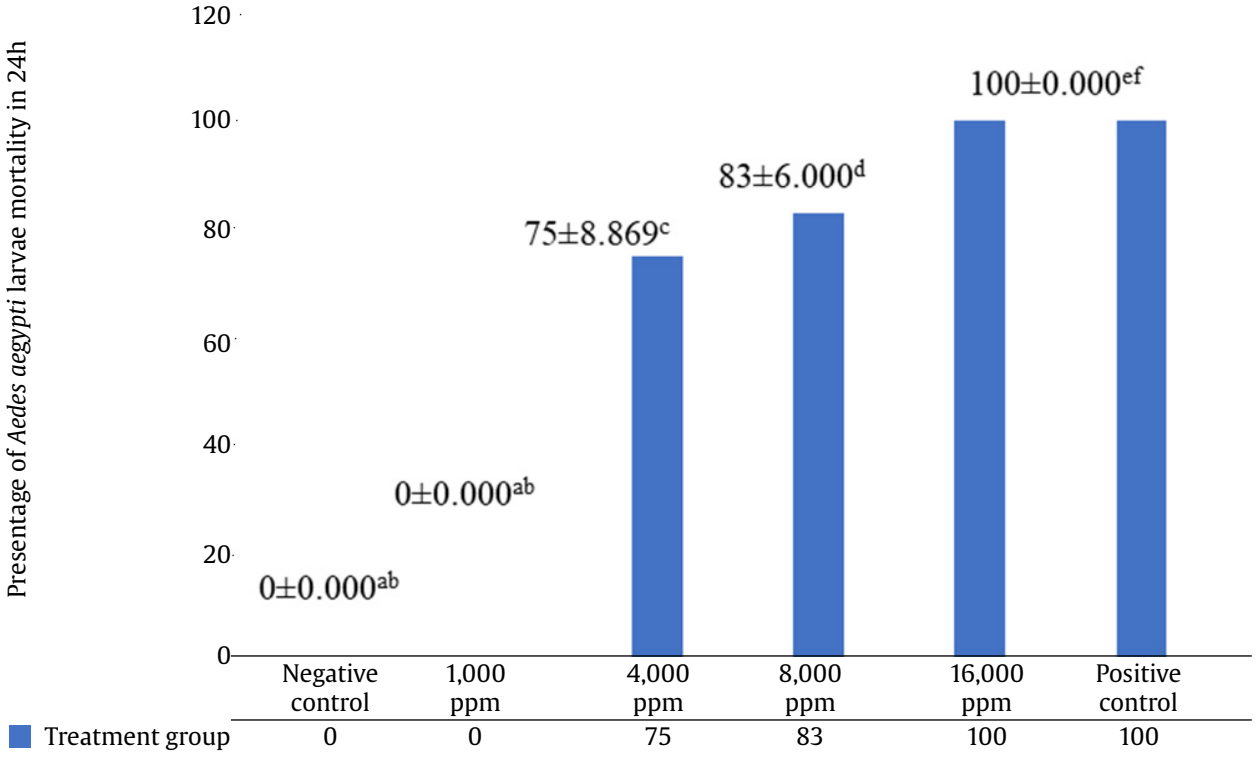

Figure 5. Percentage of *Aedes aegypti* larvae mortality in 24 h,values are mean (%) of the four-replication ± standard deviation. Different superscripts in the column indicate significance difference at P<0.05 levels

One-way Anova analysis confirmed the significance difference among the data from the treatment with different concentration.

3.3. Midgut Larvae Histopathological Studies

Under normal conditions, the gastric areas have flattened regular cells with pale clear cytoplasm. There are regular microvilli lining the apical surface that are closely attached to the basal lamina. Histopathological study showed that changes happened in the midgut epithelium of the gastric areas. At LEAE 4,000 ppm, there was no observed significant damageon the gastric area (Figure 6). There was only minor damage to the brush border, epithelial cells, nucleus and basement membrane are still visible. But, treatment with LEAE 16,000 ppmshowed swelling of the gastric caeca, damage of the brush border, the epithelial cells, and the nucleus (Table 4).

4. Discussion

Phytochemical study of LEAE showed its larvicidal bioactive compounds, particularly Oleic acid, 9-Hexadecenoic acid, and cis-1,3-Eicosenoic acid. They belong to monounsaturated fatty acids. The presence of the oleic acid in the extract from *Jatropha curcas*, as larvicidal activity is consistent with the study of Cantrell *et al.* 2011. Oleic acid has larvicidal activity against 1 d-old *Ae. aegypti* larvae, with an LD_{50} of 47.9 ppm (Cantrell *et al.* 2011). Oleic acid containing extract from Vitex species, *Citrullus colocynthis* (whole plant), *Millettia pinata* (seed), and *Avicennia marina* (mangrove), possess larvicidal properties against *Ae. aegypti* and Cx. quinquefasciatus (Cantrell *et al.* 2011; Kannathasan *et al.* 2008; Karthi *et al.* 2020; Perumalsamy *et al.* 2015; Rahuman *et al.* 2008). According to (Gurunathan *et al.* 2016), Oleic acid was

Table 3. Probit analysis of the larvicidal activity of leave ethyl acetate extract from *Aegle marmelos*

LC_{50} (ppm)	95% Confidence level		LC_{90} (ppm)	95% Confidence level	
	Lower bound	Upper bound		Lower bound	Upper bound
3,917	3,311	4,585	7,341	6,074	9,835

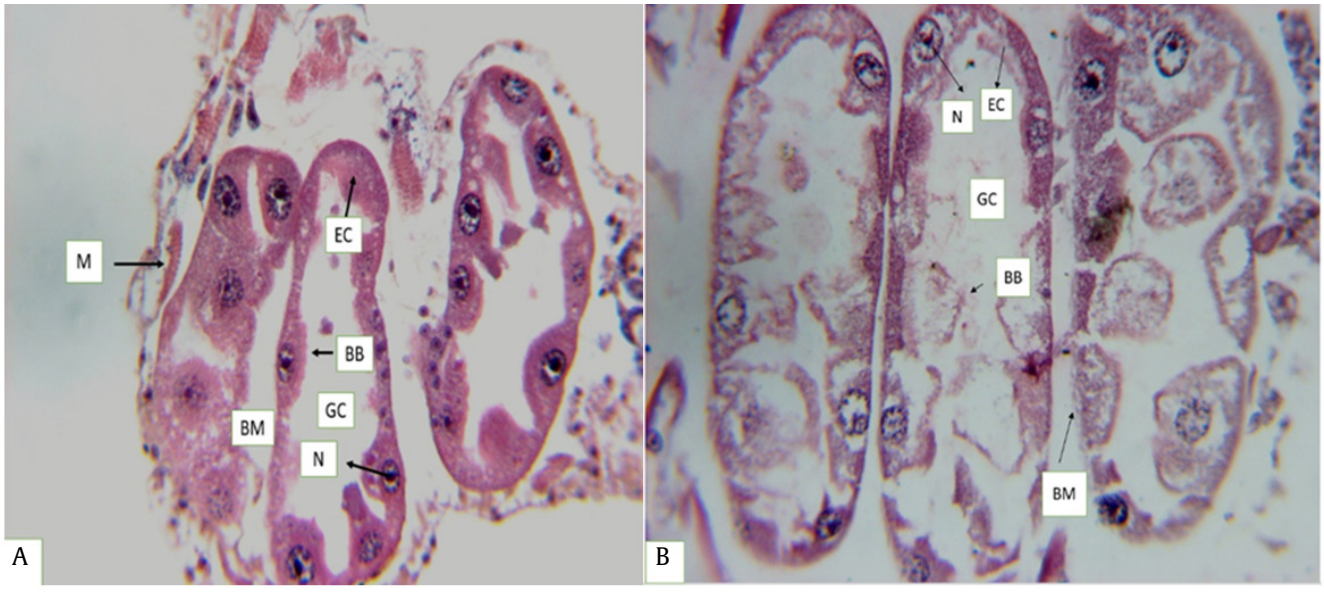

Figure 6. Longitudinal section of gastric caeca (400x) of *Ae. aegypti* after treatment with LEAE A: LEAE 4,000 ppm; B: LEAE 16,000 ppm. BB, brush border; BM, basal membrane; EC, epithelial cell; GC, gastric caeca; M, muscle; N, nucleus

Table 4. The effect of LEAE on the larvae midgut (gastric caeca) epithelium damage

	LEAE	
	4,000 ppm	16,000 ppm
EC (epithelial cell)	minor damage	damage
BB (brush border)	minor damage	damage
BM (basal membrane)	still visible	thinning
N (nucleus)	minor damage	damage

found to be the most effective larvicide against *Ae. aegypti* with LC_{50} 8.51 ppm. Oleic acid can reduce chitinase and ecdysone 20-monooxygenase activity which are most pronounced in both target species, *Ae. aegypti* and Cx. quinquefasciatus. Both enzymes are very important in insect growth and morphogenesis (Gurunathan *et al.* 2016). Oleic acid can also inhibit AchE, causing neurological activity disorders and larval death (Perumalsamy *et al.* 2015). Additionally, the other two fatty acids, 9-hexadecenoid acid and cis-1,3-Eicosanoic acid (Table 1), are known for their larvicidal and insecticidal activities (Farag *et al.*2021) (Karthi *et al.* 2020).

This study confirmed our previous study (Sari and Susilowati 2019) that LEAE from *Aegle marmelos* has an effective larvicidal effect. The LC_{50} (3,917 ppm) and LC_{90} (7,341 ppm) values of this study are weaker than our previous report with LC_{50} = 2.03% (2,030 ppm) and LC_{90}= 3.04% 3,040 ppm) (Sari and Susilowati 2019). This means that the extraction procedure and quality of the extract needs to be improved Compared with the LC_{50} and LC_{90} of various extracts (methanol and essential oils) from *Aegle marmelos* against Cx. quinquefasciatus, it is clear that the LEAE has weaker larvicidal activity.

Study on changes in the larvae midgut epithelium is very important in knowing the effectiveness of any insecticides. The midgut of insect larvae has key functions in the excretion of various digestive enzymes and absorption of nutrients (Yu *et al.* 2015). In this study, LEAE causes histopathological changes in the larvae midgut epithelium. LEAE can damage the brush borders, the nucleus and nucleolus, hypertrophy of the gastric caeca. This is consistent with several studies that have been conducted previously study that changes in larval midgut epithelial cells to mild, moderate, and severe (Firmansyah *et al.* 2019; Wang *et al.* 2019). This damage may be caused by bioactive secondary metabolite (Mujeeb *et al.* 2014) in LEAE that are oxidative stress which can cause damage to the cells that compose the larval body changes, namely the cuboidal epithelial cells that make up the cell membranes of the larva's body (Figueora *et al.* 2020). The Oleic acid and 9-Hexadecenoid acid in LEAE from *Aegle marmelos* may damage the larvae midgut of *Ae. aegypti* (Sharma *et al.* 2018).

The future use of LEAE as insecticide depends on its effective way to manage damaging *Ae. aegypti* larvae. Therefore, the ability of LEAE to damage the function of larvae midgut epithelium is a determining factor in its effectiveness as an insecticidal compound. The mode of action knowledge of the LEAE bioactive molecules that cross and damage the gut will help to find the best use of LEAE with more rational design in the future (Denecke *et al.* 2018). Further research is needed, such as a study in using pure compounds that are present in the LEAE. It is necessary to investigate the mechanism of LEAE that crosses the midgut epithelium whether through passive (diffusion) or active (transporter based, endocytosis) routes; and its interaction between fatty acid binding proteins with the unsaturated fatty acids in LEAE (Caccia *et al.* 2012).

In conclusion, the study concluded that LEAE from Aegle marmelos is a potential natural insecticide to kill *Ae. aegypti*. This is the first report about the LEAE from *Aegle. marmelos* that contains bioactive compounds that have strong larvicidal activity; has *Ae. aegypti* larvicidal activity that comparable with most frequent use insecticide, Temephos and has the ability to disturb the essential functions of larvae midgut epithelium. This study suggested that Aegle marmelos can be applied as natural insecticide to eradicate the population of *Ae. aegypti*.

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References

Abdulhafiz, F., Mohammed, A., Kayat, F., Bhaskar, M., Hamzah, Z., Podapati, S.K., Reddy, L.V., 2020. Xanthine oxidase inhibitory activity, chemical composition, antioxidant properties and GC-MS analysis of keladi candik (*Alocasia longiloba* Miq). *Molecules*. 25, 2658. https://doi.org/10.3390/molecules25112658

Al-Mehmadi, R.M., Al-Khalaf, A.A., 2010. Larvicidal and histological effects of Melia azedarach extract on *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *J King Saud Univ-Sci.* 22, 77–85. https://doi.org/10.1016/j.jksus.2010.02.004

Angajala, G., Pasupala, P., Subhashini, R., 2014. One-step biofabrication of copper nanoparticles from *Aegle marmelos* correa aqueous leaf extract and evaluation of its anti-inflammatory and mosquito larvicidal efficacy. *RSC Adv.* 4, 51459-51470. https://doi.org/10.1039/c0xx00000x

Boligon, A.A., Schwanz, T.G., Piana, M., Bandeira, R.V., Frohlich, J.K., de Brum, T.F., Athayde, M.L., 2013. Chemical composition and antioxidant activity of the essential oil of *Tabernaemontana catharinensis* A. DC. leaves. *Nat. Prod. Res*. 27, 68-71. https://doi.org/1080/14786419.2011.653971

Caccia, S., Grimaldi, A., Casartelli, M., Falabella, P., De Eguileor, M., Pennacchio, F., Giordana, B., 2012. Functional analysis of a fatty acid binding protein produced by Aphidius ervi teratocytes. *J Insect Physiol.* 58, 621-627. https://doi.org/10.1016/j.jinsphys.2011.12.019

Cantrell, C.L., Ali, A., Duke, SO., Khan, I., 2011. Identification of mosquito biting deterrent constituents from the Indian folk remedy plant *Jatropha curcas. J Med Entomol.* 48, 836-845. https://doi.org/10.1603/ME10244

Denecke, S., Swevers, L., Douris, V., Vontas, J., 2018. How do oral insecticidal compounds cross the insect midgut epithelium? *Insect Biochem Mol Biol.* 103, 22-35. https://doi.org/10.1016/j.ibmb.2018.10.005

Eldawud, R., Wagner, A., Dong, C., Gupta, N., Rojanasakul, Y., O'Doherty, G., Dinu, C.Z., 2020. Potential antitumor activity of digitoxin and user-designed analog administered to human lung cancer cells. *Biochim Biophys Acta Gen Subj*. 1864, 129683. doi:10.1016/j.bbagen.2020.129683

Ershad, M., Meredith, A., Shah, N., Khalid, M.M., 2021. Cardioactive Steroid Toxicity. In StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC.

Farag, S.M., Essa, E.E., Alharbi, S.A., Alfarraj, S., Abu El-Hassan, G.M.M., 2021. Agro-waste derived compounds (flax and black seed peels): Toxicological effect against the West Nile virus vector, *Culex pipiens* L. with special reference to GC–MS analysis. *Saudi J Biol Sci.* 28, 5261–5267. https://doi.org/10.1016/j.sjbs.2021.05.038

Figueroa, D., Signore, A., Araneda, O., Contretas, H.R., Concha, M., Garcia, C., 2020. Toxicity and differential oxidative stress effects on zebrafish larvae following exposure to toxins from the okadaic acid group. *J Toxocol Environ Heal.* 83, 573-588. https://doi.org/10.1080/15287394.2020.1793046

Firmansyah, N.E., Aulung, A., Wibowo, H., Subahar, R., 2019. Activity of *Ocimum sanctum* leaf extract against Aedes aegypti larvae: midgut histopathological alteration. *ASPIRATOR-J Vector-borne Dis Stud.* 11, 13–18. https://doi.org/10.22435/asp.v11i1.215

Freitas, C.S., Lage, D.P., Oliveira-da-Silva, J.A., Costa, R.R., Mendonça, D.V.C., Martins, V.T., Coelho, E.A.F., 2021. *In vitro* and *in vivo* antileishmanial activity of β-acetyl-digitoxin, a cardenolide of Digitalis lanata potentially useful to treat visceral leishmaniasis. *Parasite*. 28, 38. https://doi.org/10.1051/parasite/2021036

Gurunathan, A., Senguttuvan, J., Paulsamy, S., 2016. Evaluation of mosquito repellent activity of isolated oleic acid, eicosyl ester from *Thalictrum javanicum. Indian J Pharm Sci*. 78, 103–110. https://doi.org/10.4103/0250-474x.180259

Guz, L., Wawrzykowski, J., Adaszek, Ł., 2021. Anti-babesial potential and chemical composition of essential oil from yarrow *Achillea millefolium. Pol. J. Vet. Sci.* 24, 79-84. https://doi.org/10.24425/pjvs.2021.136795

Johnson, T.O., Odoh, K.D., Nwonuma, C.O., Akinsanmi, A.O., Adegboyega, A.E., 2020. Biochemical evaluation and molecular docking assessment of the anti-inflammatory potential of *Phyllanthus nivosus* leaf against ulcerative colitis. *Heliyon*. 6, e03893. https://doi.org/10.1016/j.heliyon.2020.e03893

Kamaraj, C., Bagavan, A., Elango, G., Zahir, A.A., Rajakumar, G., Marimuthu, S., Rahuman, A.A., 2011. Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus. Indian. J. Med. Res*. 134, 101-106.

Kannathasan, K., Senthilkumar, A., Venkatesalu, V., Chandrasekaran, M., 2008. Larvicidal activity of fatty acid methyl esters of Vitex species against *Culex quinquefasciatus. Parasitol Res*. 103, 999-1001. https://doi.org/10.1007/s00436-008-1078-1

Karthi, S., Vinothkumar, M., Karthic, U., Manigandan, V., Saravanan, R., Vasantha-Srinivasan, P., Kamaraj, Chinnaperumal, Shivakumar, M.S., 2020. Biological effects of *Avicennia marina* (Forssk.) vierh. extracts on physiological, biochemical, and antimicrobial activities against three challenging mosquito vectors and microbial pathogens. *Environ Sci Pollut Res Int.* 27, 15174-15187. https://doi.org/10.1007/s11356-020-08055-1

Manimegalai, K., Annapoorani, C.A., 2013. *Aegle marmelos* as an effective agent against the fourth instar larvae of *Culex quinquefasciatus* (Diptera) : Culicidae). *Int Res J Pharm Appl Sci.* 3, 64–68.

Moni, S.S., Alam, M.F., Sultan, M.H., Makeen, H.A., Alhazmi, H.A., Mohan, S., Anwer, T., 2021. Spectral analysis, *in vitro* cytotoxicity and antibacterial studies of bioactive principles from the leaves of *Conocarpus lancifolius*, a common tree of Jazan, Saudi Arabia. *Braz J Biol*, 83, e244479. https://doi.org/10.1590/1519-6984.244479

Mujeeb, F., Bajpai, P., Pathak, M., 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos. Biomed Res Int.* 2014, 1–11. https://doi.org/10.1155/2014/497606

Okoye, F.B., Osadebe, P.O., Nworu, C.S., Okoye, N.N., Omeje, E.O., Esimone, C.O., 2011. Topical anti-inflammatory constituents of lipophilic leaf fractions of *Alchornea floribunda* and *Alchornea cordifolia. Nat. Prod. Res.* 25, 1941-1949. https://doi.org/10.1080/14786419.2010.512272

Patel, A.R., Garach, D., Chakraborty, M., Kamath, J.V, 2012. *Aegle marmelos* (Linn.): A therapeutic boon for human health. *Int J Res Ayurveda Pharm.* 3, 159–163.

Perumalsamy, H., Jang, M.J., Kim, J.R., Kadarkarai,M., Ahn, Y.J., 2015. Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species. *Parasites and Vectors*. 8, 2-14. https://doi.org/10.1186/s13071-015-0848-8

Poochi, S.P., Easwaran, M., Balasubramanian, B., Anbuselvam, M., Meyyazhagan, A., Park, S., Kaul, T., 2020. Employing bioactive compounds derived from *Ipomoea obscura* (L.) to evaluate potential inhibitor for SARS-CoV-2 main protease and ACE2 protein. *Food Front.* 1, 168-179. https://doi.org/10.1002/fft2.29

Prakash, N.K.U., Sripriya, N.S., Raj, D.D., Deepa, S., Bhuvaneswari, S., 2019. Antioxidant potency and GC-MS composition of *Origanum majorana* Linn. *Pak J Pharm Sci*. 32, 2117-2122.

Rahuman, A.A., Venkatesan, P., Gopalakrishnan, G., 2008. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Cittrullus colocynthis* (Linn.) Schrad. *Parasitol Res*. 103, 1383-1390. https://doi.org/10.1007/s00436-008-1146-6.

Reegan, A.D., Gandhi, M.R., Paulraj, M.G., Ignacimuthu, S., 2015. Ovicidal and oviposition deterrent activities of medicinal plant extracts against *Aedes aegypti* L. and *Culex quinquefasciatus* say mosquitoes (Diptera: Culicidae). *Osong Public Heal Res Perspect.* 6, 64–69. https://doi.org/10.1016/j.phrp.2014.08.009

Sari, M., Susilowati, R.,2019. Efektivitas ekstrak daun maja (*Aegle marmelos* (L) Corr) sebagai larvasida *Aedes aegypti. Jurnal Kedokteran YARSI.* 27, 001–009.

Sarma, R., Mahanta, S., Khanikor, B., 2017. Insecticidal activities of the essential oil of *Aegle marmelos* (Linnaeus, 1800) against *Aedes aegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (say, 1823). *Univers J Agric Res.* 5, 304–311. https://doi.org/10.13189/ujar.2017.050508

Shah, S.I.A., Tipu, M.Y., Aslam, A., Khan, A.U., Shafee, M., Khan, S.A., Akbar, A., 2021. Elucidating antiviral activity of *Curcuma longa* against H9 N2 influenza virus using embryonated chicken egg model. *Trop Biomed*. 38, 353-359. https://doi.org/10.47665/tb.38.3.078

Sharma, A., Kumar, S., Tripathi, P., 2018. Effects of *Achyranthes aspera* extracts on thesurvival and midgut histo-architecture of *Aedes aegypti* L. early IV Instars. *The Open Parasitology Journal*. 6, 41–51. https://doi.org/10.2174/1874421401806010041

Thakur, R.S., Ahirwar, B., 2019. A steroidal derivative from *Trigonella foenum* graecum L. that induces apoptosis *in vitro* and *in vivo. J. Food. Drug. Anal*. 27. 231-239. https://doi.org/10.1016/j.jfda.2018.05.001

Wang, Z., Perumalsamy, H., Wang, X., Ahn, Y.J., 2019. Toxicity and possible mechanisms of action of honokiol from Magnolia denudata seeds against four mosquito species. *Sci Rep.* 9, 1–19. https://doi.org/10.1038/s41598-018-36558-y

Yu, K.X., wong, C.L., Ahmad, R., Jantan, I., 2015. Larvicidal activity, inhibition effect on development, histopathological alteration and morphological aberration induced by seaweed extracts in Aedes aegypti (Diptera: Culicidae). *Asian Pac J Trop Med.* 8, 1006–1012. https://doi.org/10.1016/j.apjtm.2015.11.011