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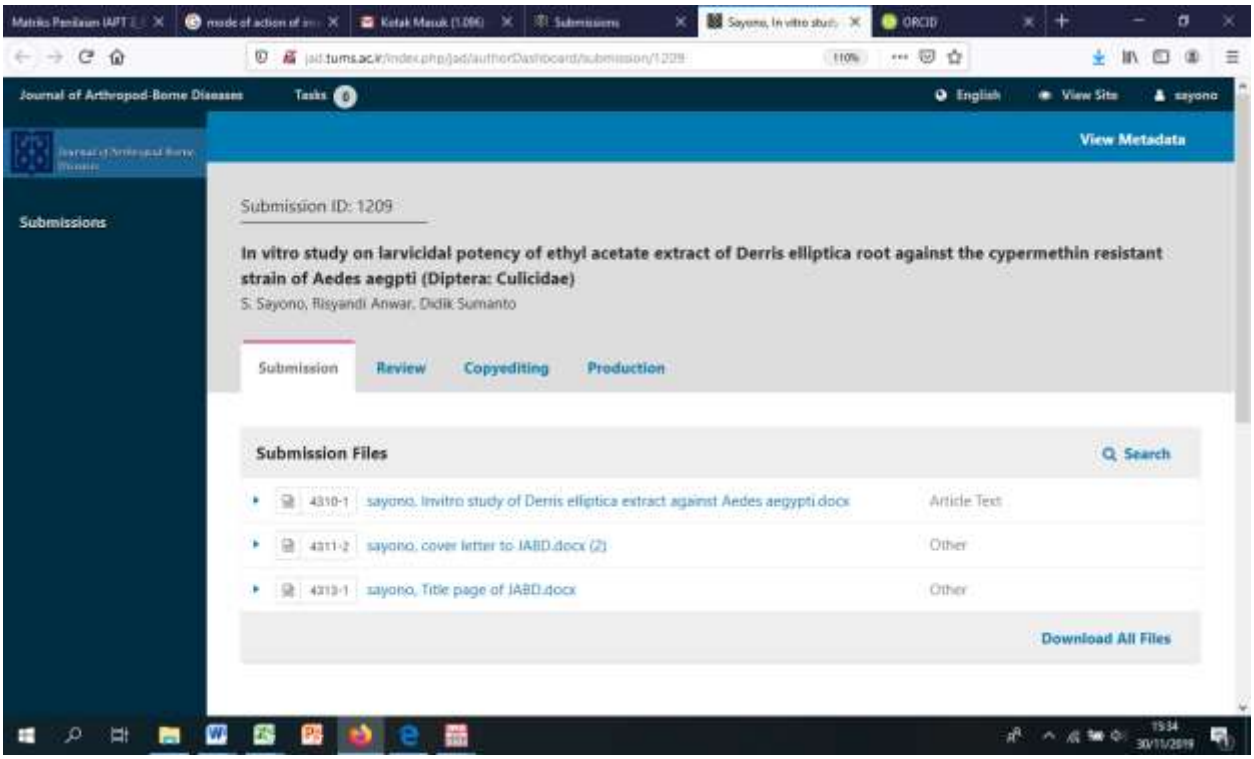

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Semarang, 30 November 2019

The Editor-in-Chief: Journal of Arthropod-Borne Diseases

Dear Sir,

Attached, please find our manuscript entitled:

In vitro study on larvicidal potency of ethyl acetate extract of *Derris elliptica* root against the cypermethin resistant strain of *Aedes aegpti* (Diptera: Culicidae)

which we would like to submit to the scientific journal that you run as a short communication.

Information on the exploration and evaluation of larvicidal activity of various plant extracts continues to grow, including research findings on *Derris elliptica* extracts. We explored the local species of this plant in an effort to obtain the bioactive compound for larvicide formulation, as an alternative effort to solve the problem of Dengue vector resistance to temephos. We would like to share our preliminary data that might be important in providing scientific information to develop the supporting material for the Dengue vector control in Indonesia.

We do believe that the manuscript would fill the data unavailability and also very much relevant to your reader.

I am looking forward to hearing your favorable reply

Sincerely yours,
S. Sayono
On behalf of the authors

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Title page

In vitro study on larvicidal potency of ethyl acetate extract of Derris elliptica root against the cypermethin resistant strain of Aedes aegpti (Diptera: Culicidae)

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In vitro study on larvicidal potency of ethyl acetate extract of *Derris elliptica* root against the cypermethrin resistant strain of *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Background: *Derris elliptica* extracts have a high larvicidal potential against the laboratory strain of *Aedes aegypti* larvae, but the effect on offspring larvae of pyrethroid-resistant strains of the species is lack understood. This study aimed to determine the larvicidal activity of the ethyl acetate extract of tuba root against the offspring larvae of cypermethrin resistant *Aedes aegypti*.
Methods: The experimental study occupied four levels of ethyl acetate extract of *Derris elliptica* namely 10, 25, 50, and 100 ppm, and each level was four times replicated. As many as twenty of healthy third instar larvae of the cypermethrin resistant offspring of *Aedes aegypti* were subjected to each experiment group. Larval mortality rate and lethal concentration 50% subject (LC50) were calculated after 24 and 48 hours of exposure time.
Results: Mortality of larvae increased directly proportional to the increase of extract concentration. Larval mortality rates after 24 and 48 hours of exposure were 40 – 67.5 % and 62.5 – 97.5%, and LC50 were 34.945 and 6.461 ppm, respectively.
Conclusion: The ethyl acetate extract of *Derris elliptica* has larvicidal potential against *Aedes aegypti* larvae of the cypermethrin resistant offspring. Isolation of the specific compound is necessarily done to obtain the active ingredient for larvicide formulation.
Keywords: larvicidal potential, ethyl acetate extract, *Derris elliptica* root, cypermethrin resistant, *Aedes aegypti*

Introduction

The resistance of *Aedes aegypti* larvae to temefos inhibits the eradication of the Dengue vector, and intrigues researchers to find the other active ingredients as the alternatives. Natural chemical compounds (1), including *Derris elliptica* roots (2), are interesting to study for several reasons including but not limited to readily degraded and there is no bioaccumulation in the environment (3). Researchers have proven that *Derris elliptica* extracts have high larvicidal potential against the laboratory strain larvae of *Aedes aegypti* (1,2,4,5). However, when methanol and ethanol extracts of Derris elliptica were exposed to the filed-caught or the temephos resistant larvae of *Aedes aegypti* showed a lower larvicidal potential (6,7). We are exploring the tuba root larvicidal activities of various extract types, both high polar, polar, semi-polar and nonpolar with water, methanol, ethyl acetate, and n-hexane solvents. Initial studies showed that ethyl acetate extract effectively killed *Aedes aegypti* larvae which were susceptible to temephos 0.02 ppm (8).

The results of monitoring of dengue vector susceptibility in Central Java, Indonesia show a wide spread of resistance to cypermethrin 0.05% (9), as occurs in various Dengue endemic areas in other countries (10). Cypermethrin is one of the active ingredients of pyrethroid insecticide which has caused knockdown resistance (kdr) (11). This resistance mechanism was indicated with the target site insensitivity in the voltage-gated sodium channel (VGSC) gene (12. The mechanism of action of cypermethrin is different from temephos. Temephos is an organophosphate insecticide class, which inhibited the acetylcholinesterase enzyme (13).

The lethal effects of various phytochemicals contained in *Derris elliptica* root extracts against the offspring larvae of the cypermethrin-resistant strain mosquitoes are still lack understood and are interesting to be investigated. This study aimed to determine the larvicidal activity of ethyl acetate extract of *Derris elliptica* root against the offspring larvae of the cypermethrin-resistant strain of *Aedes aegypti*.

Material and methods

This experiment is the early part of an ongoing study 'Isolation of specific compounds of *Derris elliptica* as the larvicidal ingredients against *Aedes aegypti* mosquito in the Dengue control'. Ethyl acetate extract is the last step of the sequential extraction process (14,15). In summary modification, the extraction process started by maceration of the tuba root powder in methanol solvent for 3x24 hours, and then filtered. The clean part of the liquid is evaporated and produced the methanol extract (the crude extract). Furthermore, the crude extract was partitioned liquid-liquid with n-hexane solvent to bind the nonpolar lead compounds and resulted in water fraction and n-hexane fraction. The water fraction obtained was partitioned with ethyl acetate to bind the semi-polar lead compounds and produced the water fraction and ethyl acetate fraction. All fractions produced were evaporated by using a rotary evaporator to produce four types of extracts, including the ethyl acetate extract which was first completed.

The subjects of this study were the offspring filial 2 (F2) larvae of the cypermethrin 0.05% resistant strain of *Aedes aegypti*. The parental mosquitoes were the F1 larvae of the *Aedes aegypti* that is reared from F0 larvae obtaining from a household survey in the Dengue endemic areas in the Community Health Center of Kedung Mundu, Tembalang District, Semarang City. Larvae were maintained in the Epidemiology and Tropical Diseases laboratory of Public Health Faculty of Universitas Muhammadiyah Semarang, Indonesia. The larvae were placed on a plastic tray containing tap water. Conditions of temperature and humidity were maintained in the range of $28^0C \pm 2^0C$ and 75% ± 10%. The larvae were fed dog food. Bioassay tests apply the WHO standard procedures for larvicide testing (16). There are three important parts at this stage, namely preparation of extract concentration, selection of research subjects, and exposure of research subjects with various *Derris Elliptica* extracts. The concentration of the bioassay test used a range of 10, 25, 50, and 100 ppm, the effective concentration in another study using *Aedes aegypti* larvae of laboratory strains (8). The subject of the research was the third instar larvae of *Aedes aegypti*, in intact condition, and actively moving. As many as twenty larvae were subjected to each experiment group.

Two control groups, negative (tap water) and positive (temephos 0.02 ppm) control were followed. The effectiveness of the larvicidal activity of the ethyl acetate extract of *Derris elliptica* root was determined by the LC50 that was obtained from probit analysis. Analysis data was performed descriptively and analytically by using SPSS statistical software version 15.0. The research protocol obtained ethical approval from the Ethics Committee of Health Research of Public Health Faculty of Universitas Muhammadiyah Semarang with registration number 231/KEPK-FKM/UNIMUS/2019.

Results

The ethyl acetic extracts of *Derris elliptica* root showed the larvicidal activity against the *Aedes aegypti* larvae of offspring from the resistant parental to cypermethrin adulticide. There was an increase in the larval mortality rate of *Aedes aegypti* larvae after 24 and 48 hours of exposure to the ethyl acetic extract from 40-67.5% to 62.5-97.5% (Table 1), with LC50 of

34.945 and 6.461 ppm, respectively (Table 2). The larval mortality rate increased directly with the extract concentration. There were no larvae died in the negative control, and 100% of larvae died in the positive control. The trend of the knockdown larvae showed that the slow larvicidal activity of the ethyl acetic extract of *Derris elliptica* root to the F2 larvae of cypermethrin resistant *Aedes aegypti* (Fig.1).

Discussion

Results of the experiment showed that the ethyl acetic extracts of *Derris ellitpica* have a high larvicidal effect against the offspring of the cypermethrin resistant strain of *Aedes aegypti*. At the concentration of 100 ppm, the larvicidal effect of the semi-polar extract type is still below the temephos 0.02 ppm. This condition shows that the larvicidal potency of the ethyl acetic extract of *Derris elliptica* root is still five thousand times lower than temephos. Nevertheless, these preliminary data and information have given a new hope that this extract can be an alternative ingredient of larvicide to inhibit the growth of *Aedes aegypti* larvae, even to the strains that are already resistant to cypermethrin adulticide.

In this experiment, three insecticide compounds were compared, namely cypermethrin, tuba root extract, and temephos. Ethyl acetic extract of tuba root and temephos larvicides be able to cause high mortality of the *Aedes aegypti* offspring larvae from cypermethrin resistant strains. This condition showed that there is still a way to eradicate the Dengue vector, even from strains that have been resistant to other insecticides because each insecticide compound has a different mode of action (17,18). Cypermethrin is a compound of the pyrethroid class, an insecticide group that disrupts the function of sodium channels in insect nerves (19). Under normal conditions, the voltage-gated sodium channel (VGSC) gene works ‘open’ and ‘close’ to regulate electrical impulses into the cell. The linkage of the pyrethroid insecticide molecule to the gene disrupts the nerve regulation and impulse of the nerve flowing continuously so that the insects become convulsion and died (20). However, if the point mutations occur in this gene, the linkage of the pyrethroid insecticide molecule does not affect the life of the insect, and this condition caused the kdr (21).

The ethyl acetic extract of *Derris elliptica* root has a larvicidal effect against the third instar larvae, the offspring of the cypermethrin resistant strain of *Aedes aegypti*. This condition indicated that this extract has a different mechanism of action than adult cypermethrin. The *Derris elliptica* extract contains several lead compounds such as tannins, phlobatannins, terpenoids, cardiac glycosides, and flavonoids (22) mainly rotenone and rotenoids (23). Mode of action of rotenone is inhibition the cellular respiration, while pyrethrins the active compound of the pyrethroid insecticide has a mode of action in disruption of the sodium and potassium ions exchange (24). On the other hand, the effectiveness of temefos larvicide in killing the offspring larvae of the cypermethrin resistant strain of *Aedes aegypti* proved that rotational insecticide with different modes of action and targets is important to do. Temefos is a compound that plays a role in protein carbonylation so that it causes the general oxidative damage in larvae of insects (25).

The maximum effect of the ethyl acetate extract of *Derris elliptica* was achieved at 48 hours of exposure time. This condition indicated that the mode of action of this extract is slower than temephos. It can be understood that temephos is a pure chemical compound, while the plant extract still contains many chemical compounds, which may have antagonistic effects (26). Extraction with ethyl acetate solvent has selected chemical compounds that are semi-polar, according to the nature of the solvent. However, the extraction results still allow the dissolution of many plant chemical compounds with various modes of action although flavonoid was the

dominant compound (27). Therefore, the pure compounds from these extracts that have the best larvicidal activity are necessarily understood.

Conclusion

The ethyl acetate extract of *Derris elliptica* root has a high larvicidal activity against offspring larvae of the cypermethrin resistant strain of *Aedes aegypti*, although the effect is slower than temephos. Isolation of the pure compounds of the extract is needed to find the specific active compounds for larvicide formulation.

Acknowledgment

The authors wish to thank President of Universitas Muhammadiyah Semarang for the research permission for conducting the experiment in laboratory study at the Epidemiology and Tropical Diseases laboratory; Dean of Mathematical and Natural Sciences Faculty of Universitas Garut, West Java, Indonesia; Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education for the funding of the study.

References

1. Dohutia C, Bhattacharyya DR, Sharma SK, Mohapatra PK, Bhattacharjee K, Gogoi K, Gogoi P, Mahanta J, Prakash A (2015) Larvicidal activity of few select indigenous plants of North East India against disease vector mosquitoes (Diptera: Culicidae). Trop Biomed. 32(1):17-23. https://www.ncbi.nlm.nih.gov/pubmed/25801251
2. Komalamisra N, Trongtokit Y, Rongsriyam R, Apiwathnasorn C (2005) Screening for larvacidal activity in some Thai plants against four mosquito vector species. Southeast Asian J. Trop. Med. Public Health. 36(6): 1412-1422.
3. Arnason JT, Sims SR, Scott IM (2012) Natural products from plants as insecticides. In: Phytochemistry and Pharmacognosy. Encyclopedia of Life Support System (EoLSS). Available: https://www.eolss.net/Sample-Chapters/C06/E6-151-13.pdf
4. Zubairi SI, Sarmidi MR, Aziz RA (2015) A preliminary study on mosquito larvicidal efficacy of rotenone extracted from Malaysia Derris sp. Jurnal Teknologi. 76(1):275-279. Available on www.jurnalteknologi.utm.my
5. Komansilan A, Suriani NW, Lawalata H (2017) Test toxic tuba root extract as a natural insecticide on larvae of Aedes aegypti mosquito vector of Dengue fever. Int. J. Chemtech *Res.* 10(4): 522-528. http://sphinxsai.com/2017/ch_vol10_no4/3/(522528)V10N4CT.pdf
6. Sayono, Nurullita U, Suryani M (2010) Pengaruh konsentrasi flavonoid dalam ekstrak akar tuba (Derris elliptica) terhadap kematian larva Aedes aegypti. J. Kesehat. Masy. Indones. 2010;6(1):38-47.
7. Sayono S, Permatasari A, Sumanto D (2019) The Effectiveness of *Derris elliptica* (Wall.) Benth Root Extract Against Temephos-resistant *Aedes aegypti* Larvae. IOP Conference Series: Earth and Environmental Science. 292:102052. doi:10.1088/1755-1315/292/1/ 012052.
8. Sayono S, Hidayati APN, Fahri S, Sumanto D, Dharmana E, Hadisaputro S, Asih PBS, Syafruddin D (2016) Distribution of Voltage-Gated Sodium Channel (Nav) Alleles among

the Aedes aegypti Populations In Central Java Province and Its Association with Resistance to Pyrethroid Insecticides. PLoS One. 11(3): e0150577.

9. Sayono S, Anwar R, Sumanto D (2019) Isolation of specific compounds of *Derris elliptica* as the larvicidal ingredients against *Aedes aegypti* mosquito in the Dengue control. An ongoing research project report. Unpublished data.
10. Moyes C, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, Raghavendra K, Pinto J, Corbel V, David JP, Weetman D (2017) Contemporary status of insecticides resistance in the major Aedes vectors of arboviruses infecting humans. PLoS Negl Trop Dis. 11(7): e0005625. Doi: 10.1371/journal.pntd.0005625
11. Singh AK, Tiwari MN, Prakash O, Sing MP (2012) A current review of Cypermethrin-induced neurotoxicity and nigrostriatal dopaminergic neurodegeneration. Neuropharmacology 10:64-71. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3286848/pdf/CN-10-64.pdf
12. Liu N, Xu Q, Zhu F, Zhang L (2006) Pyrethroid resistance in mosquitoes. Insect Science. 13:159-166. DOI 10.1111/j.1744-7917.2006.00078.x.
13. Insecticide Resistance Action Committee (2019) IRAC Mode of Action Classification Scheme. www.irac-online.org
14. Liu Z (2008) Preparation of botanical samples for biomedical research. Endocr Metab Immune Disord Drug Targets. 8(2):112–121. https://www.ncbi.nlm.nih.gov/pmc/articles/P-MC3936020/pdf/nihms556279.pdf
15. Li XJ, Hareyama T, Tezuka Y, Zhang Y, Miyahara T, Kadota S (2005) Five New Oleanolic Acid Glycosides from *Achyranthes bidentata* with Inhibitory Activity on Osteoclast Formation. Planta Med. 71(7):673–679. doi:10.1055/s-2005-871275
16. World Health Organization (2005) Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization: 7-12. https://apps.who.int/iris/handle/10665/69101
17. Das SK (2013) Mode of action of pesticides and the novel trends – A critical review. International Research Journal of Agricultural Science and Soil Science 3(11):393-401. DOI: http:/dx.doi.org/10.14303/irjas.2013.118
18. Spark TC, Nauen R (2015) IRAC: Mode of action classification and insecticide resistance management. Pestic Biochem Physiol. 121: 122-128. doi: 10.1371/journal.pone.0170836. https://www.sciencedirect.com/science/article/pii/S0048357514002272
19. Soderlund DM (2012) Molecular Mechanisms of Pyrethroid Insecticide Neurotoxicity: Recent Advances. Arch Toxicol. 86(2): 165–181. doi:10.1007/s00204-011-0726-x.
20. Silver KS, Du Y, Nomura Y, Olivera EE, Salgado VL, Zhoro BS, Dong K (2014) Voltage-Gated Sodium Channels as insecticide targets. Adv In Insect Phys. 46: 389–433. doi:10.1016/B978-0-12-417010-0.00005-7
21. Soderlund DM, Knipple DC (2003) The molecular biology of knockdown resistance to pyrethroid insecticides. Insect Biochem Mol Biol. 33(6):563-577. doi:10.1016/s0965-1748(03)00023-7

22. Lagunay RAE, Uy MM (2015) Evaluation of the phytochemical constituents of the leaves of Ficus minahassae Tesym & De Vr., Casuarina equisetifolia Linn., Leucosyke capitellata (Pior) Wedd., Cassia sophera Linn., Derris elliptica Benth., Cyperus brevifolius (Rottb.) Hassk., Piper abbreviatum Opiz., Ixora chinensis Lam., Leea aculeata Blume, and Drymoglossum piloselloides Linn. AAB Bioflux 7(1):51-58. http://www.aab.bioflux.com.ro/docs/2015.51-58.pdf
23. Zubairi SI, Ramli KA, Majid FAA, Sarmidi MR, Aziz Ra (2005) Biological screening on the extract of Derris elliptica. Proceeding of KUSTEM 4th ANNUAL SEMINAR ON Sustainability Science and Management, 2nd - 3rd May 2005, Primula Beach Resort, Kuala Terengganu. http://eprints.utm.my/id/eprint/5263/
24. Ghosh A, Chowdhury N, Chandra G (2012) Plant extracts as potential mosquito larvicides. Indian J Med Res. 135:581-598. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3401688/
25. Rodríguez-Cavallo E, Guarnizo-Méndez J, Yépez-Terrill A, Cárdenas-Rivero A, Díaz-Castillo F, Méndez-Cuadro D (2018) Protein carbonylation is a mediator in larvicidal mechanisms of *Tabernaemontana cymosa* ethanolic extract. J King Saud Univ Sci. 31(4):464-471. https://doi.org/10.1016/j.jksus.2018.04.019
26. Millugo TK, Osoma LK, Ochanda JO, Owuor BO, Wamunyokoli FA, Oyugi JO, Ochieng JW (2013) Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (Rauvolfia caffrasond.): further evidence to support biotechnology in traditional medicinal plants. BMC Complementary and Alternative Medicine 13:285. doi:10.1186/1472-6882-13-285
27. Thavamoney N, Sivanadian L, Tee LH, Khoo HE, Prasad KN, Kong KW (2018) Extraction and recovery of phytochemical components and antioxidative properties in fruit parts of Dacryodes rostrata influenced by different solvents. J Food Sci Technol. 55(7):2523–2532. https://doi.org/10.1007/s13197-018-3170-6

Tables 1

Concentration (ppm)	24-hours mortality rate (%)			48-hours mortality rate (%)		
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**
0 (dw)	0	0	0	0	0	0
10	30	40	35	60	65	62,5
25	40	40	40	80	90	85
50	40	70	55	90	100	95
100	65	70	67,5	95	100	97,5
0.02 (tem)	100	100	100	100	100	100

dw = distilled water; tem = temephos

Table 2. The LC50 of larvicidal activity of the Derris elliptica ethyl acetate extract against offspring larvae of the cypermethrin-resistant *Aedes aegypti*

Exposure time (hours)	**Regression equation**	**LC50 (95% confidence limits)**
24	Y= -1.331+0.862X	34.945 (18.179 – 70.780)
48	Y= -1.419+1.751X	6.461 (2.206 – 10.359)

Figures

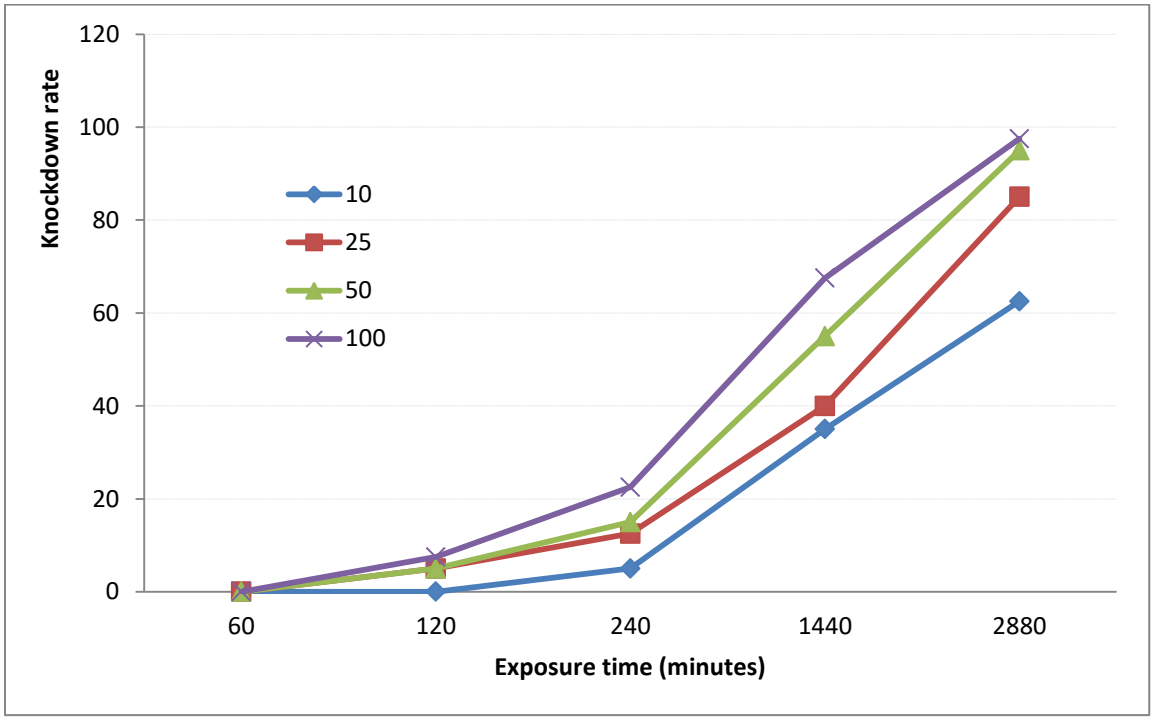

Figure 1. The trend of larval knockdown rate in each extract concentration based on the exposure time. The colored-line represents the concentrations of the extract.

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Dear Dr Sayono,

Thank you for submitting the manuscript entitled: “In Vitro Study on Larvicidal Potency of Ethyl Acetate Extract of *Derris elliptica* Root against the Cypermethrin Resistant Strain of *Aedes aegypti* (Diptera: Culicidae)” by: Sayono Sayono; Risyandi Anwarand Didik Sumanto.

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Yours sincerely,

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Best regard,

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Comments status

Ref A:

comment	Status/your reply
Comment 1:	
Comment 2:	
Comment …:	
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Ref C:

comment	Status/your reply
Comment 1: Please change the title of the article	The old title: **In Vitro Study on Larvicidal Potency of Ethyl Acetate Extract of *Derris elliptica* Root against the Cypermethrin Resistant Strain of *Aedes aegypti* (Diptera: Culicidae)** The new title: **Larvicidal Activity of Ethyl Acetate Extract of *Derris elliptica* Root against the Third-instar Larvae of Cypermethrin-resistant *Aedes aegypti* offspring**
Comment 2: Please write for the table above and there is no explanation	Table 1 Larvicidal activity of the ethyl acetate extract type of *Derris elliptica* against the third-instar larvae of cypermethrin-resistant *Aedes aegypti* offspring (see page 8)
Comment 3: In the results section, please explain more about the knockdown results of age 2 larvae	Longer exposure (48 hours) of the extract showed that the mortality of *Ae. aegypti* larvae was almost twice that of 24 hours exposure (Table 1). Line 20-21 page 3.
Comment 4: Please use newer references. Use more from 2017-2020.	We retain some old references (before 2017) for reasons of substance relevance. We also added some new references in list number 12, 17, 21, and 34.
Comment 5: In the results section, the findings of Temephos and Cypermethrin on the larvae of Aedes aegypti are not given, while in the discussion of the article, it is reminded that the work has been done safely. Please mention the findings.	Result of cypermethrin bioassay test was stated in Method section (lines 36-38, page 2)
Comment 6: The discussion of the study is incomplete and the results of the study have not been compared with the results of similar	We have updated the discussion by adding relevant references (lines 31-33, 35-39, 41-45 on page 3; 3-4 on page 4), as well as updating library number 34 (line 13 on page 4).

studies	
Comment 7: Please correct the typing errors	typing erros is fixed
Additional improvements:	- Explanation of the research subject (... the third-instar larvae of Cypermethrin-resistance Ae. aegypti offspring) in the Background Abstract (lines 10-11 page 1). - Editorial changes to sentences in paragraph 1 Introduction (lines 30-32, 37-41 page 1; lines 3-11 page 2).

Comments status

Ref A:

There was no written comment from Ref A, but we made improvements based on the highlights contained in the review form A, combined with comments of Ref C.

comment	**Status/your reply**
Comment 1: Inadequate introduction	We have improved the sentences in Introduction section (yellow highlighted) on lines 30-32 and 37-41 on page 1, and lines 3-11 and 14-15 on page 2.
Comment 2: Poorly described methods	We have added an explanation of the measurement of the resistance status of the study subjects and their results in lines 35-38 on page 2 and lines 5-7 on page 3 (Material and method section).
Comment 3: Inadequate discussion	In the Discussion section, we've added more discussion descriptions (lines 29-33, 35-39, and 41-45 on page 3, and lines 3-4 on page 4), with newer references.
Other revisions:	
1. Reference	Replacement of the newer references (numbers 12, 17, 21, and 34).
2. Change of title according to comments Ref C	The new title: **Larvicidal Activity of Ethyl Acetate Extract of *Derris elliptica* Root against the Third-instar Larvae of Cypermethrin-resistant *Aedes aegypti* offspring**
3. Additional research data	Longer exposure (48 hours) of the extract showed that the mortality of *Ae. aegypti* larvae was almost twice that of 24 hours exposure (Table 1). Line 20-21 page 3.
4. Typing errors	We have improved all parts of the manuscript

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Journal of Arthropod-Borne Diseases <jad@tums.ac.ir> 20 Juli 2020 pukul 13.36
Kepada: say.epid@gmail.com

Dear Dr Sayono,

Enclosed you will find the comments of reviewers on your manuscript (**No.1209**) entitled**: "In Vitro Study on Larvicidal Potency of Ethyl Acetate Extract of *Derris elliptica* Root against the Cypermethrin Resistant Strain of *Aedes aegypti* (Diptera: Culicidae)"** as an attached file. Could you read it carefully and correct the article within 7 days please? Please answer to the comments (one by one) in a separate file as well. Looking forward to hearing from you at your earliest convenience.

Yours sincerely,

Prof. H. Vatandoost

Editor-in-Chief

Journal of Arthropod Borne Diseases

5 lampiran

- **comment status.doc** 48K
- **In Vitro Study on ....doc** 117K
- **review form A.doc** 250K
- **A. In Vitro Study on ....doc** 145K
- **review form C.doc** 253K

Sayono Sayono <say.epid@gmail.com> 20 Juli 2020 pukul 14.59
Kepada: Journal of Arthropod-Borne Diseases <jad@tums.ac.ir>

Thank you very much, Prof. Hasan Vatandoost for this information. We will immediately respond to this information according to the timeframe allocated.

Best regard,

Sayono
Department of Epidemiology and Tropical Diseases
School of Public Health of Universitas Muhammadiyah Semarang
Jalan Kedung Mundu Raya 18, Semarang, 50273
Indonesia

[Kutipan teks disembunyikan]

Sayono Sayono <say.epid@gmail.com> 28 Juli 2020 pukul 09.37
Kepada: Journal of Arthropod-Borne Diseases <jad@tums.ac.ir>

Dear Prof. Hasan Vatandoost
Editor-in-Chief of JABD

My manuscript (No.1209) titled: "In Vitro Study on Larvicidal Potency of Ethyl Acetate Extract of Derris Elliptica Root Against the Cypermethrin Resistant Strain of Aedes aegypti (Diptera: Culicidae)", at first I submitted it as "**Short Communication**". But the reviewers classified it into **Original paper**, so I had to revise more. I ask for 2-3 additional days to complete this revision. thanks.
Best regard,

S. Sayono
Department of Epidemiology and Tropical Diseases
School of Public Health of Universitas Muhammadiyah Semarang
Jalan Kedung Mundu Raya 18, Semarang, 50273
Indonesia

[Kutipan teks disembunyikan]

Sayono Sayono <say.epid@gmail.com> 8 Agustus 2020 pukul 18.28
Kepada: Journal of Arthropod-Borne Diseases <jad@tums.ac.ir>

Dear Prof. Hasan Vatandoost
Editor-in-Chief of JABD

Attached is the revised version of my manuscript number 1209 entitled: **Larvicidal Activity of Ethyl Acetate Extract of Derris elliptica Root against the Third-instar Larvae of Cypermethrin-resistant Aedes aegypti offspring,** and comment status of reviewers and revision details. I hope that the results of this revision can continue to the next process.

Best regard,
S. Sayono
Department of Epidemiology and Tropical Diseases
School of Public Health of Universitas Muhammadiyah Semarang
Jalan Kedung Mundu Raya 18, Semarang, 50273
Indonesia

[Kutipan teks disembunyikan]

2 lampiran

cover letter to JABD - revised version.docx
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A. In Vitro Study on ...final-rev-882020.doc
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Journal of Arthropod-Borne Diseases <jad@tums.ac.ir> 31 Oktober 2020 pukul 01.11
Kepada: Sayono Sayono <say.epid@gmail.com>

Dear Dr Sayono,

Please reply to each comment in the attached table.

Yours sincerely,

J Arthropod-Borne Diseases
[Kutipan teks disembunyikan]

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Journal of Arthropod-Borne Diseases <jad@tums.ac.ir> 1 November 2020 pukul 23.41
Kepada: Sayono Sayono <say.epid@gmail.com>

Dear Dr Sayono,

Please reply to each comment in the attached table.

Yours sincerely,
J Arthropod-Borne Dis

[Kutipan teks disembunyikan]

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Journal of Arthropod-Borne Diseases <jad@tums.ac.ir> 1 November 2020 pukul 23.43
Kepada: say.epid@gmail.com

Dear Dr Sayono ,

Please reply to each comment in the attached table.

Yours sincerely,
J Arthropod-Borne Dis

On 2020-08-08 15:58, Sayono Sayono wrote:

[Kutipan teks disembunyikan]

3 lampiran

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Sayono Sayono <say.epid@gmail.com> 3 Desember 2020 pukul 11.13
Kepada: Journal of Arthropod-Borne Diseases <jad@tums.ac.ir>

Dear Editor-in-Chief of Journal of Arthropod-Borne Diseases

We attached this revision of the manuscript (No.1209) entitled: "**In Vitro Study on Larvicidal Potency of Ethyl Acetate Extract of Derris elliptica Root against the Cypermethrin Resistant Strain of Aedes aegypti (Diptera: Culicidae)**" According to reviewer's comments, we have done a lot changes to the manuscript, including the title, changed to: **Larvicidal Activity of Ethyl Acetate Extract of Derris elliptica Root against the Third-instar Larvae of Cypermethrin-resistant Aedes aegypti offspring.**
We hope that these changes are in accordance with the quality standards of this journal. Thank you very much.

Best regards,
Sayono
Department of Epidemiology and Tropical Diseases
School of Public Health of Universitas Muhammadiyah Semarang
Jalan Kedung Mundu Raya 18, Semarang, 50273

Indonesia

[Kutipan teks disembunyikan]

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UNIVERSITAS MUHAMMADIYAH SEMARANG
FACULTY OF PUBLIC HEALTH

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Semarang, 30 November 2020

The Editor-in-Chief: Journal of Arthropod-Borne Diseases

Dear Sir,

Attached, please find our revised version manuscript number 1209 entitled (new title):

Larvicidal Activity of Ethyl Acetate Extract of Derris elliptica Root against the Third-instar Larvae of Cypermethrin-resistant Aedes aegypti offspring

which we would like to submit to the scientific journal that you run as an original paper (based on the reviewer suggestions).

We have revised the manuscript according to the reviewers' suggestions, and added one figure from the data analysis. We hope that these additions will clarify the results of this study.

We do believe that the manuscript would fill the data unavailability and also very much relevant to your reader.

I am looking forward to hearing your favorable reply

Sincerely yours,
S. Sayono
On behalf of the authors

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Larvicidal Activity of Ethyl Acetate Extract of *Derris elliptica* Root against the Third-instar Larvae of Cypermethrin-Resistant *Aedes aegypti* Offspring

Sayono Sayono; Risyandi Anwar; Didik Sumanto

[1]Faculty of Public Health, Universitas Muhammadiyah Semarang, Semarang, Indonesia
[2]Herbal medicine research of Dentistry Faculty, Universitas Muhammadiyah Semarang, Semarang, Indonesia
[3]Laboratory of Epidemiology and Tropical Diseases, Faculty of Public Health, Universitas Muhammadiyah Semarang, Semarang, Indonesia

*Corresponding author**:** Dr Sayono Sayono, E-mail: say.epid@gmail.com

Abstract

Background: *Derris elliptica* extracts have a high larvicidal potential against the laboratory strain of *Aedes aegypti* larvae, but the effect on offspring larvae of pyrethroid-resistant strains of the species is lack understood. This study aimed to determine the larvicidal activity of the ethyl acetate extract of tuba root against the third-instar larvae of the Cypermethrin-resistant *Ae. aegypti* offspring.

Methods: The experimental study occupied four levels of ethyl acetate extract of *D. elliptica* namely 10, 25, 50, and 100 ppm, and each level was four times replicated. As many as twenty of healthy third-instar larvae, offspring of Cypermethrin-resistant *Ae. aegypti* were subjected to each experiment group. Larval mortality rate and lethal concentration 50% subject (LC_{50}) were calculated after 24 and 48 hours of exposure time.

Results: Mortality of larvae increased directly proportional to the increase of extract concentration. Larval mortality rates after 24 and 48 hours of exposure were 40–67.5% and 62.5–97.5%, and LC_{50} were 34.945 and 6.461 ppm, respectively.

Conclusion: The ethyl acetate extract of *D. elliptica* has the high effectiveness larvicidal potential against the third-instar larvae, offspring of the Cypermethrin-resistant *Ae. aegypti*. Isolation of the specific compound is necessarily done to obtain the active ingredient for larvicide formulation.

Keywords: Larvicidal activity, Ethyl acetate extract, *Derris elliptica* root, Cypermethrin resistant, *Aedes aegypti*

Introduction

The resistance of *Ae. aegypti* to several pyrethroid and organophosphate insecticide compounds such as deltamethrin, lambda cyhalothrin, cypermethrin, malathion, and temephos (1, 2) inhibits the public health action in eradicating the dengue vector, and intrigues researchers to find the other active ingredients as the alternatives. Natural chemical compounds (3), including *D. elliptica* roots (4), are interesting to study for several reasons including but not limited to readily degraded and there is no bioaccumulation in the environment (5). Researchers have proven that *D. elliptica* extracts have high larvicidal potential against the laboratory strain larvae of *Ae. aegypti* (3, 4, 6, 7). However, when methanol extract of *D. elliptica* was exposed to the filed-caught larvae of *Ae. aegypti* showed a lower larvicidal potential (8). This fact showed that the

different extract types of the tubal root have different effects against the different strains of *Ae. aegypti* larvae where the field-caught larvae were more resistant to the phytochemical compound.

The results of monitoring of dengue vector susceptibility in Central Java, Indonesia showed a wide spread of resistance to cypermethrin 0.05% (1), as occurs in various Dengue endemic areas in other countries (4, 9, 10). Cypermethrin is one of the active ingredients of pyrethroid class insecticide which has caused knockdown resistance (kdr) (11). This resistance mechanism was indicated with the target site insensitivity in the voltage-gated sodium channel (VGSC) gene (1, 12). The action mechanism of cypermethrin is different from the temephos. This compound is an active ingredient of organophosphate insecticide class which inhibited the acetylcholinesterase enzyme (13). This different mechanism of action is interesting to be studied in understanding the larvicidal activity spectrum of *D. elliptica* root extracts.

The main biochemical compounds of *D. elliptica* are alkaloids, flavonoids, sterols, tannins, and triterpenoids (14, 15), and rotenone is the most important of a specific compound of flavonoid (16). These compounds have a toxic effect that kills insect larvae through disrupting mechanisms of the endocrine and hormonal systems (14) and reducing the esterase and monooxygenase enzymes (16). Initial studies showed that ethyl acetate, methanol, and n-hexane extracts of *D. elliptica* that have different polarity effectively killed *Ae. aegypti* larvae which were susceptible to temephos 0.02 ppm (17), but on the other hand, the ethanol extract type has a lower effect against the temephos-resistant strains (18). The lethal effects of different specific phytochemicals contained in *D. elliptica* root extracts against the offspring larvae of the cypermethrin-resistant strain *Ae. aegypti* is still lack understood and is interesting to be studied. It is important to evaluate the larvicidal effect of the semi polar extract, ethyl acetate against this strain. This study aimed to determine the larvicidal activity of ethyl acetate extract of *D. elliptica* root against the third-instar larvae of the cypermethrin-resistant *Ae. aegypti* offspring.

Materials and Methods

This experiment is the early part of an ongoing study 'Isolation of specific compounds of *Derris elliptica* as the larvicidal ingredients against *Aedes aegypti* mosquito in the dengue control'. Ethyl acetate extract is the last step of the sequential extraction process (19, 20). In the summary modification, the extraction process started by maceration of the tuba root powder in methanol solvent for 3 x 24 hours, and then filtered. The clean part of the liquid is evaporated and produced the methanol extract (the crude extract). Furthermore, the crude extract was partitioned liquid-liquid with n-hexane solvent to bind the nonpolar lead compounds and resulted in water fraction and n-hexane fraction. The water fraction obtained was partitioned with ethyl acetate to bind the semi-polar lead compounds and produced the water fraction and ethyl acetate fraction. All fractions produced were evaporated by using a rotary evaporator to produce four types of extracts, including the ethyl acetate extract which was first completed.

The subjects of this study were the offspring filial 2 (F2) larvae of the cypermethrin 0.05% resistant strain of *Ae. aegypti*. The parental mosquitoes were the F1 larvae of the *Ae. aegypti* that is reared from F0 larvae obtaining from a household survey in the Dengue endemic areas in the Community Health Center of Kedung Mundu, Tembalang District, Semarang City, and subjected to bioassay test using the Cypermetrhin-0.05% compound. The result of the bioassay test showed a mortality rate of 85%, which indicated that the mosquito population was resistant to the pyrethroid compound (21). Larvae were maintained in the Epidemiology and Tropical Diseases

laboratory of Public Health Faculty of Universitas Muhammadiyah Semarang, Indonesia. The larvae were placed on a plastic tray containing tap water. Conditions of temperature and humidity were maintained in the range of 28±2 °C and 75±10%. The larvae were fed dog food. Bioassay tests apply the WHO standard procedures for larvicide testing (21). There are three important parts at this stage, namely preparation of extract concentration, selection of research subjects, and exposure of research subjects with various *D. elliptica* extracts. The concentration of the bioassay test used a range of 10, 25, 50, and 100 ppm, the effective concentration in another study using *Ae. aegypti* larvae of laboratory strains (17). The subject of the research was the third instar larvae offspring of the Cypermethrin-resistant *Ae. aegypti*, in intact condition, and actively moving. As many as twenty larvae were subjected to each experiment group.

Two control groups, negative (tap water) and positive (temephos 0.02 ppm) control were followed. The effectiveness of the larvicidal activity of the ethyl acetate extract of *D. elliptica* root was determined by the LC_{50} that was obtained from probit analysis. This LC_{50} will be compared with the LC_{50} of previous experiment results of the same extract type against the laboratory (susceptible) strain of *Ae. aegypti* larvae (17). Analysis data was performed descriptively and analytically by using SPSS statistical software version 15.0. The research protocol obtained ethical approval from the Ethics Committee of Health Research of Public Health Faculty of Universitas Muhammadiyah Semarang with registration number 231/KEPK-FKM/UNIMUS/2019.

Results

The ethyl acetic extracts of *D. elliptica* root showed the larvicidal activity against the *Ae. aegypti* larvae of offspring from the resistant parental to cypermethrin adulticide. There was an increase in the larval mortality rate of *Ae. aegypti* larvae after 24 and 48 hours of exposure to the ethyl acetic extract from 40–67.5% to 62.5–97.5% (Table 1), with LC_{50} of 34.945 and 6.461 ppm, respectively (Table 2). The larval mortality rate increased directly with the extract concentration. There were no larvae died in the negative control, and 100% of larvae died in the positive control. The trend of the knockdown larvae showed that the slow larvicidal activity of the ethyl acetate extract of *D. elliptica* root to the third-instar larvae, offspring F2 of cypermethrin-resistant *Ae. aegypti* (Fig. 1). Statistical analysis showed the differences in larval mortality rate based on the dosage and exposure time (Fig.2).

Discussion

Results of the experiment showed that the ethyl acetic extract of *D. ellitpica* has a high larvicidal activity against the third-instar larvae of the cypermethrin-resistant *Ae. aegypti* F2 offspring, although at the concentration of 100 ppm for 24 hours, the larvicidal effect of the ethyl acetate extract type is still lower than the larvicidal activity of temephos 0.02 ppm. Although the ethyl acetate extract type of tuba root shows lower larvicidal activity than temephos, this extract still indicates high larvicidal potential because its LC_{50} is lower than 50 ppm. A previous study reported that the effective larvicidal activity of plant extracts was categorized into three levels, namely high (LC_{50} < 50 ppm), moderate (LC_{50} < 100 ppm), and low LC_{50} < 750 ppm) (4). The results of this experiment indicate that the potency of the ethyl acetate extract of *D. elliptica* root against the third-instar larvae of cypermethrin-resistant *Ae. aegypti* F2 offspring is 84.4% lower than the same extract potency against the susceptible (laboratory) strain (17) indicating by the

LC_{50} bioassay test for 24 hours, respectively 34,945 ppm and 21,063 ppm. This extract also had a higher larvicidal potential against F2 offspring larvae of Cypermethrin-resistant *Ae. aegypti* rather than the temephos-resistant offspring (18). The larvicidal activity of this extract was also better than the methanolic extract of *M. glaziovii* peel (22), *A. pinata* (23), *T. patula* (24), *H. forskaolii* (25), *O. campechianum* and *O. quixos* (26), and *A. occidentale* (27) against the third-instar larvae of *Ae. aegypti*, although lower than the specific isolate compound of *F. vulgare* (28) and *P. aduncum* (26) essential oils, and *P. foetida* ethyl acetate extract (29). These preliminary data and information have given new hope that this extract can be an alternative ingredient of larvicide to inhibit the growth of *Ae. aegypti* larvae, even to the strains that are already resistant to cypermethrin adulticide.

These results also indicate that temephos is still effective in killing the third-instar larvae offspring F2 of the cypermethrin-resistant *Ae. aegypti*. This condition showed that there is still a way to eradicate the Dengue vector, even from strains that have been resistant to other insecticides because each insecticide compound has a different mode of action (30, 31). Cypermethrin is a compound of the pyrethroid class, an insecticide group that disrupts the function of sodium channels in insect nerves (32). Under normal conditions, the voltage-gated sodium channel (VGSC) gene works 'open' and 'close' to regulate electrical impulses into the cell. The linkage of the pyrethroid insecticide molecule to the gene disrupts the nerve regulation and impulse of the nerve flowing continuously so that the insects become convulsion and died (33). However, if the point mutations occur in this gene, the linkage of the pyrethroid insecticide molecule does not affect the life of the insect, and this condition caused the kdr (34).

The high larvicidal activity of the ethyl acetic extract of *D. elliptica* root against the third instar larvae, the offspring of the cypermethrin-resistant *Ae. aegypti* indicates that this extract has a different mechanism of action than adulticide cypermethrin. The *D. elliptica* extract contains several lead compounds such as tannins, phlobatannins, terpenoids, cardiac glycosides, and flavonoids (35) mainly rotenone and rotenoids (36). Mode of action of rotenone is inhibition the cellular respiration, while pyrethrins the active compound of the pyrethroid insecticide has a mode of action in disruption of the sodium and potassium ions exchange (37). It means that the exploration of specific isolates of *D. elliptica* extract has the opportunity to be developed into larvicidal bioactive compounds with different modes of action. On the other hand, the effectiveness of temephos larvicide in killing the offspring larvae of the cypermethrin resistant strain of *Ae. aegypti* proved that rotational insecticide with different modes of action and target sites is necessary done. Temephos is a compound that plays a role in protein carbonylation so that it causes the general oxidative damage in larval cellular of insects (38).

The maximum effect of the ethyl acetate extract of *D. elliptica* was achieved at 48 hours of exposure time. This condition indicated that the mode of action of this extract is slower than temephos. It can be understood that temephos is a pure chemical compound, while the plant extract still contains many chemical compounds, which may have antagonistic effects (39). Extraction with ethyl acetate solvent has selected chemical compounds that are semi-polar, according to the nature of the solvent. However, the extraction results still allow the dissolution of many plant chemical compounds with various modes of action although flavonoid was the dominant compound (40). Therefore, the pure compounds from these extracts that have the best larvicidal activity are necessarily understood.

Conclusion

The ethyl acetate crude extract of *D. elliptica* root has a high larvicidal activity against the third-instar larvae, offspring of the cypermethrin-resistant strain of *Ae. aegypti*, although the effect is lower and slower than temephos. Isolation of the pure compounds of the extract is needed to find the specific active compounds for larvicide formulation.

Acknowledgements

The authors wish to thank President of Universitas Muhammadiyah Semarang for the research permission for experimenting in laboratory study at the Epidemiology and Tropical Diseases laboratory; Dean of Mathematical and Natural Sciences Faculty of Universitas Garut, West Java, Indonesia; Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education for the funding of the study.

References

1. Sayono S, Hidayati APN, Fahri S, Sumanto D, Dharmana E, Hadisaputro S, Asih PBS, Syafruddin D (2016) Distribution of Voltage-Gated Sodium Channel (*Nav*) alleles among the *Aedes aegypti* populations in Central Java Province and its association with resistance to pyrethroid insecticides. PLoS One. 11(3): e0150577.
2. Araújo AP, Paiva MHS, Cabral AM, Cavalcanti AEHD, Pessoa LFF, Diniz DFA, Helvecio E, Silva EVG, Silva NM, Anastácio DB, Pontes C, Nunes V, Souza MFM, Magalhães FJR, Santos MAVM, Ayres CFJ. (2019) Screening *Aedes aegypti* (Diptera: Culicidae) populations from Pernambuco, Brazil for resistance to Temephos, Diflubenzuron, and Cypermethrin and characterization of potential resistance mechanisms. J Insec Sci. 19(3): 1–15.
3. Dohutia C, Bhattacharyya DR, Sharma SK, Mohapatra PK, Bhattacharjee K, Gogoi K, Gogoi P, Mahanta J, Prakash A (2015) Larvicidal activity of few select indigenous plants of North East India against disease vector mosquitoes (Diptera: Culicidae). Trop Biomed. 32(1): 17–23.
4. Komalamisra N, Trongtokit Y, Rongsriyam R, Apiwathnasorn C (2005) Screening for larvacidal activity in some Thai plants against four mosquito vector species. Southeast Asian J Trop Med Public Health. 36(6): 1412–1422.
5. Arnason JT, Sims SR, Scott IM (2012) Phytochemistry and pharmacognosy - Natural products from plants as insecticides. Encyclopedia of Life Support System (EoLSS). Available at: https://www.eolss.net/Sample-Chapters/C06/E6-151-13.pdf
6. Zubairi SI, Sarmidi MR, Aziz RA (2015) A preliminary study on mosquito larvicidal efficacy of rotenone extracted from Malaysia *Derris* sp. Jurnal Teknologi. 76(1): 275–279.
7. Komansilan A, Suriani NW, Lawalata H (2017) Test toxic tuba root extract as a natural insecticide on larvae of *Aedes aegypti* mosquito vector of dengue fever. Int J Chemtech Res. 10(4): 522–528.
8. Sayono S, Nurullita U, Suryani M (2010) Pengaruh konsentrasi flavonoid dalam ekstrak akar tuba (*Derris elliptica*) terhadap kematian larva *Aedes aegypti*. [in Indonesian]. J Kesehat Masy Indones. 6(1): 38–47.
9. Sayono S, Nurullita U (2016) Situasi terkini vektor dengue [*Aedes aegypti*] di Jawa Tengah. [in Indonesian]. Kemas. Jurnal Kesehatan Masyarakat. 11(2): 96–105.

10. Moyes C, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, Raghavendra K, Pinto J, Corbel V, David JP, Weetman D (2017) Contemporary status of insecticides resistance in the major *Aedes* vectors of arboviruses infecting humans. PLoS Negl Trop Dis. 11(7): e0005625.
11. Singh AK, Tiwari MN, Prakash O, Sing MP (2012) A current review of Cypermethrin-induced neurotoxicity and nigrostriatal dopaminergic neurodegeneration. Neuropharmacology 10: 64–71.
12. Kuswah RBS, Kaur T, Dykes CL, Kumar HR, Kapoor N, Sing OP (2020) A new knockdown resistance (kdr) mutation, F1534L, in the Voltage-gated Sodium Channer of *Aedes aegypti*, co-occurring with F1534C, S989P, and V1016G. Parasites Vectors. 13: 327.
13. IRAC - Insecticide Resistance Action Committee (2019) IRAC mode of action classification scheme. IRAC International Working Group. p.5-7.
14. Ge Y, Liu P, Yang R, Zhang L, Chen H, Camara I, Liu Y, Shi W (2015) Insecticidal constituents and activity of alkaloids from *Cynanchum mongolicum*. Molecules. 20: 17483–17492.
15. Khan MR, Omoloso AD, Barewai Y (2006) Antimicrobial activity of the *Derris elliptica*, *Derris indica* and *Derris trifoliata* extractives. Fitoterapia. 77(4): 327–330.
16. Visetson S, Milne M (2001) Effect of root extract from Derris (*Derris elliptica* Benth) on mortality and detoxification enzyme levels in the *Demondback* Moth larvae (*Plutella xylostella* Linn.). Kasetsart J (Nat. Sci.). 35: 157–163.
17. Sayono S, Anwar R, Sumanto D (2020) Evaluation of toxicity in four extract types of Tuba root against Dengue vector, *Aedes aegypti* (Diptera: Cullicidae) larvae. Pak J Biol Sci. 23(12): 1530–1538.
18. Sayono S, Permatasari A, Sumanto D (2019) The effectiveness of *Derris elliptica* (Wall.) Benth root extract against Temephos-resistant *Aedes aegypti* larvae. IOP Conference Series: Earth and Environmental Science. 292. The 1st International Conference on Food Science and Technology 28-29 November 2018, Universitas Muhammadiyah Semarang, Semarang, Indonesia. 102052.
19. Liu Z (2008) Preparation of botanical samples for biomedical research. Endocr Metab Immune Disord Drug Targets. 8(2): 112–121.
20. Li XJ, Hareyama T, Tezuka Y, Zhang Y, Miyahara T, Kadota S (2005) Five new oleanolic acid glycosides from *Achyranthes bidentata* with inhibitory activity on osteoclast formation. Planta Med. 71(7): 673–679.
21. World Health Organization (2016) Monitoring and managing insecticide resistance in *Aedes* mosquito populations. Interim guidance for entomologists. The WHO Department of Control of Neglected Tropical Diseases and Global Malaria Programme, Geneva.https://www.who.int/csr/resources/publications/zika/insecticide-resistance/en/
22. Sayono S, Safira FA, Anwar R. (2019) In-vitro study on the larvicidal activity of *Manihot glaziovii* peel extract against *Aedes aegypti* larvae. Ann Parasitol. 65(4): 403–410.
23. Ravi R, Zulkrnin NSH, Rozhan NN, Nik Yusoff NR, Mat Rasat MS, Ahmad MI, et al. (2018) Chemical composition and larvicidal activities of *Azolla pinnata* extracts against *Aedes* (Diptera: Culicidae). PLoS One. 13(11): e0206982.
24. Krzyzaniak LM, Antonelli-Ushirobira TM, Panizzon G, Luiza Sereia AL, Souza JRP, Zequi JAC, Novello CR, Lopes GC, Medeiros DC, Silva DB, Leite-Mello EVS, Mello JCP (2017) Larvicidal activity against *Aedes aegypti* and chemical characterization of the

inflorescences of *Tagetes patula*. Evid Based Complement Alternat Med. Article ID 9602368. https://doi.org/10.1155/2017/9602368
25. Sillo AJ, Makirita WE, Swai H, Chacha M (2019) Larvicidal activity of *Hypoestes forskaolii* (Vahl) R.Br root extracts against *Anopheles gambiae* Giless.s, *Aedes aegypti* L, and *Culex quinquefasciatus* Say. J Exp Pharmacol. 11: 23–27.
26. Scalvenzi L, Radice M, Toma L, Severini F, Boccolini D, Bella A, Guerrini A, Tacchini M, Sacchetti G, Chiurato M, Romi R, Luca MD (2019) Larvicidal activity of *Ocimum campechianum*, *Ocotea quixos* and *Piper aduncum* essential oils against *Aedes aegypti*. Parasite 26(23): 1–8.
27. Amado CJRR, Souto CRNP, Magalhães MS, Arranz CJCE, Carvalho CJCT (2017) Chemical composition and larvicidal activity of cashew nutshell ethanolic extract against mosquito larvae. Rev Cub Quim. 29(3): 330–340.
28. Rocha DK, Matos O, Novo MT, Figueiredo AC, Delgado M, Moiteiro C (2015) Larvicidal activity against *Aedes aegypti* of *Foeniculum vulgare* essential oils from Portugal and Cape Verde. Nat. Prod. Commun. 10 (4): 677–682.
29. Rollando and Hariono M (2016) Aktivitas larvasida ekstrak etanol, fraksi n-heksan, etil asetat, dan metanol daun sembukan terhadap larva nyamuk *Aedes aegypti* dan *Anopheles* instar III. Trad Med J. 21(3): 137–142.
30. Das SK (2013) Mode of action of pesticides and the novel trends – A critical review. Int Res J Agric Sci Soil. 3(11): 393–401.
31. Spark TC, Nauen R (2015) IRAC: Mode of action classification and insecticide resistance management. Pestic Biochem Physiol. 121: 122–128.
32. Soderlund DM (2012) Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. Arch Toxicol. 86(2): 165–181.
33. Silver KS, Du Y, Nomura Y, Olivera EE, Salgado VL, Zhoro BS, Dong K (2014) Voltage-Gated Sodium Channels as insecticide targets. Adv In Insect Phys. 46: 389–433.
34. Dong K, Du Y, Rinkevich F, Nomura Y, Xu P, Wang L, Silver K, Zhorov BS (2014) Molecular Biology of Insect Sodium Channels and Pyrethroid Resistance. Insect Biochem Mol Biol.50:1-17. doi: 10.1016/j.ibmb.2014.03.012.
35. Lagunay RAE, Uy MM (2015) Evaluation of the phytochemical constituents of the leaves of *Ficus minahassae* Tesym & De Vr., *Casuarina equisetifolia* Linn., *Leucosyke capitellata* (Pior) Wedd., *Cassia sophera* Linn., *Derris elliptica* Benth., *Cyperus brevifolius* (Rottb.) Hassk., *Piper abbreviatum* Opiz., *Ixora chinensis* Lam., *Leea aculeata* Blume, and *Drymoglossum piloselloides* Linn. AAB Bioflux. 7(1): 51–58.
36. Zubairi SI, Ramli KA, Majid FAA, Sarmidi MR, Aziz RA (2005) Biological screening on the extract of *Derris elliptica*. Proceeding of Kustem 4th Annual Seminar on Sustainability Science and Management, 2005 May 2-3, Primula Beach Resort, Kuala Terengganu. Available at: http://eprints.utm.my/id/eprint/5263/
37. Ghosh A, Chowdhury N, Chandra G (2012) Plant extracts as potential mosquito larvicides. Indian J Med Res. 135: 581–598.
38. Rodríguez-Cavallo E, Guarnizo-Méndez J, Yépez-Terrill A, Cárdenas-Rivero A, Díaz-Castillo F, Méndez-Cuadro D (2018) Protein carbonylation is a mediator in larvicidal mechanisms of *Tabernaemontana cymosa* ethanolic extract. J King Saud Univ Sci. 31(4): 464–471.
39. Millugo TK, Osoma LK, Ochanda JO, Owuor BO, Wamunyokoli FA, Oyugi JO, Ochieng JW (2013) Antagonistic effect of alkaloids and saponins on bioactivity in the quinine

tree (*Rauvolfia caffrasond*.): further evidence to support biotechnology in traditional medicinal plants. BMC Compl Alternative Med. 13: 285.
40. Thavamoney N, Sivanadian L, Tee LH, Khoo HE, Prasad KN, Kong KW (2018) Extraction and recovery of phytochemical components and antioxidative properties in fruit parts of *Dacryodes rostrata* influenced by different solvents. J Food Sci Technol. 55(7): 2523–2532.

Table 1. Larvicidal activity of the ethyl acetate extract type of *Derris elliptica* against the third-instar larvae of cypermethrin-resistant *Aedes aegypti* offspring

Concentration (ppm)	24-hours mortality rate (%)			48-hours mortality rate (%)		
	Min	Max	Mean	Min	Max	Mean
0 (dw)	0	0	0	0	0	0
10	30	40	35	60	65	62.5
25	40	40	40	80	90	85
50	40	70	55	90	100	95
100	65	70	67.5	95	100	97.5
0.02 (tem)	100	100	100	100	100	100

dw= distilled water; tem= temephos

Table 2. The LC_{50} of larvicidal activity of the *Derris elliptica* ethyl acetate extract against offspring larvae of the cypermethrin-resistant *Aedes aegypti*

Exposure time (hours)	Regression equation	LC_{50} (95% confidence limits)
24	Y= -1.331+0.862X	34.945 (18.179–70.780)
48	Y= -1.419+1.751X	6.461 (2.206–10.359)

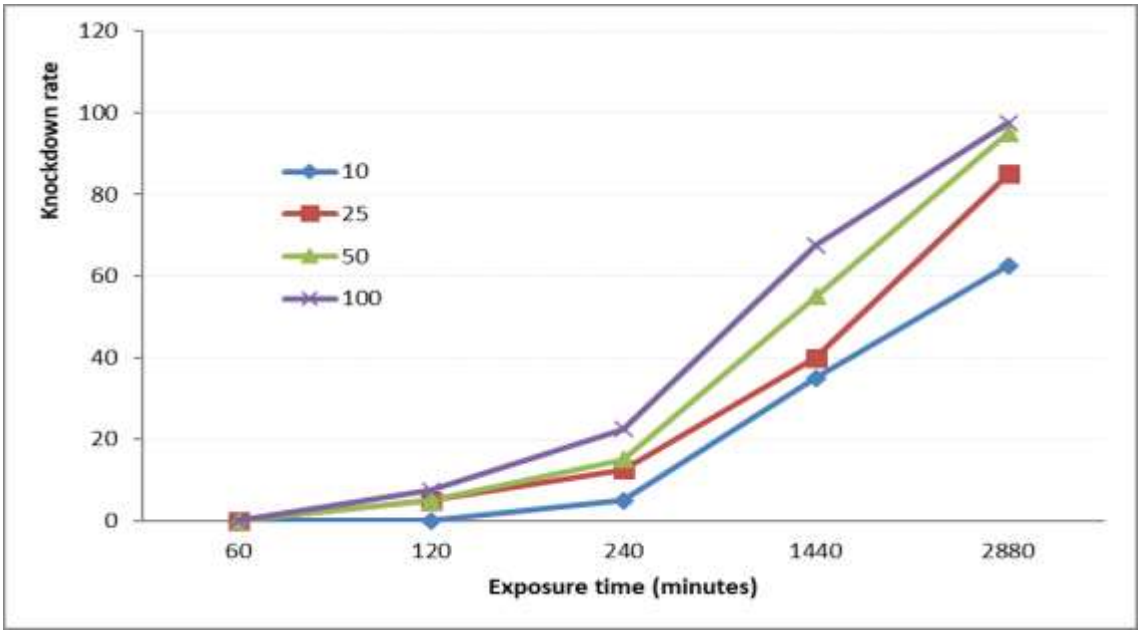

Fig. 1. The trend of larval knockdown rate in each extracts concentration based on the exposure time. The colored-line represents the concentrations of the extract

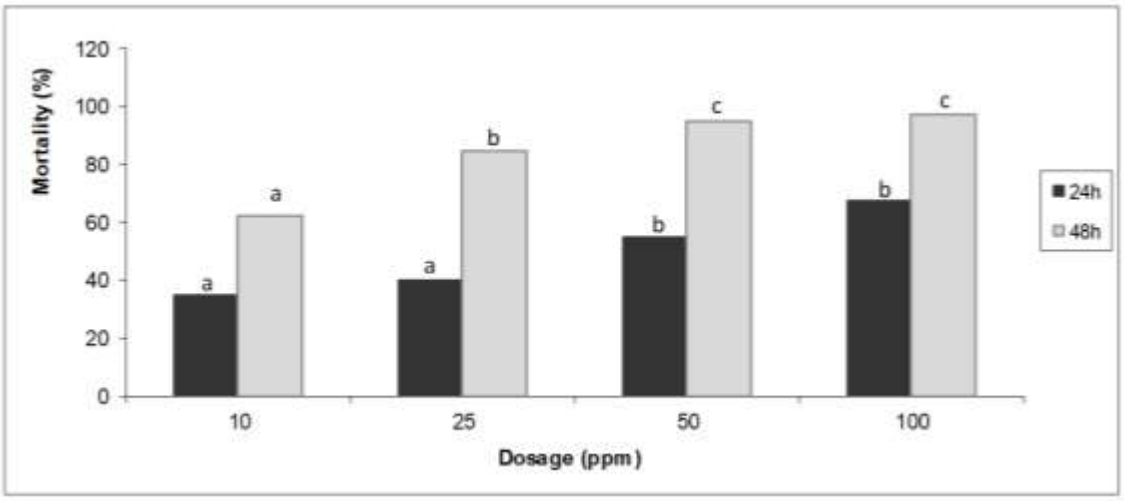

Fig. 2. Mortality rate comparison of *Aedes aegypti* larvae after 24 h and 48 h exposure of ethyl acetate extract of *Derris elliptica* root. The differences of larval mortality rate are indicated by the letters a, b and c.

Gmail **Sayono Sayono <say.epid@gmail.com>**

J Arthropod-Borne Dis,

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Journal of Arthropod-Borne Diseases <jad@tums.ac.ir> 3 Desember 2020 pukul 17.58
Kepada: say.epid@gmail.com

Dear Dr **Sayono**,

Please reply to each comment in the attached table (ref A).

Yours sincerely,

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Sayono Sayono <say.epid@gmail.com> 4 Desember 2020 pukul 23.16
Kepada: Journal of Arthropod-Borne Diseases <jad@tums.ac.ir>

We submit this revised result of manuscript number 1209 entitled Larvicidal Activity of Ethyl Acetate Extract of Derriselliptica Root against the Third-instar Larvae of Cypermethrin-resistant Aedes aegypti offspring, along with comments from Ref A and Ref C.

Sayono
Department of Epidemiology and Tropical Diseases
School of Public Health of Universitas Muhammadiyah Semarang
Jalan Kedung Mundu Raya 18, Semarang, 50273
Indonesia

[Kutipan teks disembunyikan]

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Journal of Arthropod-Borne Diseases <jad@tums.ac.ir> 1 Januari 2021 pukul 01.27
Kepada: say.epid@gmail.com

Dear Dr Sayono,

We are pleased to inform you that your revised article entitled "**Larvicidal Activity of Ethyl Acetate Extract of Derris elliptica Root against the Third-instar Larvae of Cypermethrin-Resistant Aedes aegypti Offspring**" by: **Sayono Sayono; Risyandi Anwar and Didik Sumanto** has been accepted for publication in Journal of Arthropod-Borne Diseases. Thank you for sharing with us your valuable work and looking forward to receiving your other interesting articles.

Yours sincerely,

Prof. H. Vatandoost

Editor-in-Chief

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Journal of Arthropod-Borne Diseases <jad@tums.ac.ir> 1 Januari 2021 pukul 04.48
Kepada: say.epid@gmail.com

Dear Dr Sayono,

Please find in attachment the final proof of your article entitled "**Larvicidal Activity of Ethyl Acetate Extract of *Derris elliptica* Root against the Third-instar Larvae of Cypermethrin-Resistant *Aedes aegypti* Offspring**". Pleasecheck it completely and confirm it for printing within 72 hours. In case of not receiving any reply, it will be issued assuming your final confirmation.

Yours sincerely,

Prof. H. Vatandoost

Editor-in-Chief

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Kepada: "didik.24272" <didik.24272@gmail.com>, risyandi anwar <riezdrgms@gmail.com>

Alhamdulillah.....semoga mjd awalan yg baik di tahun 2021

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Dari: **Journal of Arthropod-Borne Diseases** <jad@tums.ac.ir>
Date: Jum, 1 Jan 2021 01:27

Subject: J Arthropod-Borne Dis,
To: <say.epid@gmail.com>

Dear Dr Sayono,

We are pleased to inform you that your revised article entitled "**Larvicidal Activity of Ethyl Acetate Extract of Derris elliptica Root against the Third-instar Larvae of Cypermethrin-Resistant Aedes aegypti Offspring**" by: **Sayono Sayono; Risyandi Anwar and Didik Sumanto** has been accepted for publication in Journal of Arthropod-Borne Diseases. Thank you for sharing with us your valuable work and looking forward to receiving your other interesting articles.

Yours sincerely,

Prof. H. Vatandoost

Editor-in-Chief

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Pak Manto Unimus <didik.24272@gmail.com> 1 Januari 2021 pukul 06.17
Kepada: Sayono Sayono <say.epid@gmail.com>

Alhamdulillah.....

On Fri, Jan 1, 2021, 06:13 Sayono Sayono <say.epid@gmail.com> wrote:

> Alhamdulillah.....semoga mjd awalan yg baik di tahun 2021
>
> ---------- Forwarded message ---------
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> Date: Jum, 1 Jan 2021 01:27
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> **Dear Dr Sayono,**
>
> We are pleased to inform you that your revised article entitled "**Larvicidal Activity of Ethyl Acetate Extract of Derris elliptica Root against the Third-instar Larvae of Cypermethrin-Resistant Aedes aegypti Offspring**" by: **Sayono Sayono; Risyandi Anwar and Didik Sumanto** has been accepted for publication in Journal of Arthropod-Borne Diseases. Thank you for sharing with us your valuable work and looking forward to receiving your other interesting articles.
>
> **Yours sincerely,**
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> **Prof. H. Vatandoost**
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> **Editor-in-Chief**
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> **Journal of Arthropod Borne Diseases**

Sayono Sayono <say.epid@gmail.com> 2 Januari 2021 pukul 15.14
Kepada: Journal of Arthropod-Borne Diseases <jad@tums.ac.ir>

We have agreed with the final proof of our manuscript. Thank you very much.

Sayono
Department of Epidemiology and Tropical Diseases
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Indonesia

Pada tanggal Jum, 1 Jan 2021 pukul 04.49 Journal of Arthropod-Borne Diseases <jad@tums.ac.ir> menulis:

> **Dear Dr Sayono,**
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> Please find in attachment the final proof of your article entitled "**Larvicidal Activity of Ethyl Acetate Extract of *Derris elliptica* Root against the Third-instar Larvae of Cypermethrin-Resistant *Aedes aegypti* Offspring**". Pleasecheck it completely and confirm it for printing within 72 hours. In case of not receiving any reply, it will be issued assuming your final confirmation.
>
> **Yours sincerely,**
>
> **Prof. H. Vatandoost**
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> **Editor-in-Chief**
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