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~~Toxicity~~ Evaluation of Toxicity in Four Extract Types of Tuba Root against Dengue Vector, *Aedes aegypti* [Diptera:Culicidae] Larvae

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Abstract

Background and Objectives: Since the Dengue virus spreads rapidly and the vector becomes resistant to insecticides and larvicides, exploration of new compounds which overcome resistance problems, are easily degraded, and do not lead to bioaccumulation, is needed. This study evaluated four extract types of *Derris elliptica* represented the polar, semi-polar, and nonpolar extract against the 3rd-instar larvae of *Ae. aegypti*, and determined the effective concentration among the extracts. **Material and Methods:** The crude extract was obtained from the maceration of root powder of the plant with methanol, and subsequently evaporated. The crude extract was diluted in distilled water and partitioned sequentially with ethyl-acetate, n-hexane, and water to obtain their fractions. All the fractions were evaporated to obtain their extract types. Initial bioassay test of the extracts with concentration ranges of 50, 100, 500, and 1,000 mg/l against *Ae. aegypti* larvae ~~were done according to WHO procedure,~~ and resulted in 86-100% larval mortality rates at concentrations of 50 and 100 mg/l, except for water extract. The lower concentration range of 3, 5, 10, 25, 50, and 100 mg/l of three extract types were tested. **Results:** Larval mortality rates of 18.4–100%, 1.6–99.2%, and 0.8–98.4% with LC_{50} of 4.088, 14.066, and 21.063 mg/l respectively for n-hexane, methanol, and ethyl-acetate. FTIR analysis indicated nine lead compounds in which rotenone and ceramides were observed in all extract types. **Conclusion:** The n-hexane extract showed the highest larvicidal toxicity, and its specific compounds are necessarily isolated to obtain pure bioactive ingredients.

Keywords: larvicidal toxicity, *Derris elliptica*, *Aedes aegypti*, tuba root extract, n-hexane

Introduction

Since the Dengue virus spread rapidly in the past five decades from nine countries in 1970 to 128 countries in the tropics and subtropics recently[1,2], community attention and involvement in Dengue endemic areas in controlling *Aedes aegypti* mosquitoes has increased[3,4]. Aedes mosquito larvae become a strategic target in the Dengue vector control, where temephos rely upon larvicides. The campaign to use temephos is done seriously following and combining with the other methods to control the adult and larval stage of Aedes mosquitoes. This phenomenon occurs widely and intensively in endemic Dengue areas throughout the world for a long time[5] and results in the emergence of resistant strains of *Ae. aegypti* larvae against temephos, which have been reported in many countries[6], including in Indonesia[7,8].

The development of *Aedes aegypti* larvae resistance to temephos has hampered the Dengue vector control program. This condition triggers ~~scientists~~ researcher to find the new chemical compounds that are effective, biodegradable, and do not cause bioaccumulation in environment[9]. In line with these efforts, the utilization of the potential for tubal root toxicity has evolved from traditional to modern methods in solving the problem of controlling dengue.

Tuba (*D. elliptica* (Wallich) Benth) is a poisonous vine that is easily found on uncultivated agricultural land. This plant grows in the South Asian, Southeast Asian and Hawaiian regions[10]. Traditionally, the tuba roots have long been used by residents of the regions as a fish poison and plant pest pesticide[11,12]. The use of tuba root is related to chemical compounds contained in the plant comprising isoflavonoids[13], flavonoids[14,15], ceramides and polyhydroxy acids[16], as well as rotenoids[17] which include compounds such as rotenone, deguelin, toxicarol, sumatrol, elliptone, and malaccol[18-21].

Previously, the studies on the larvicidal toxicity of tuba root extract against *Ae. aegypti* larvae rapidly develop in several regions to find the new active compound of larvicide. A study in Thailand found that tuba root extract with petroleum ether (PE) and methanol solvents showed a different toxicity, where the PE extract showed the lower lethal concentration of 50% of mortality (LC_{50}) and LC_{90} rather than the others, namely 11.17 and 27.74 mg/l[22]. Two other studies in two different countries tested the *D. elliptica* root extract that was resulted from a combination of two solvents. In Malaysia, a 1:1 combination of methyl-chloride and methanol results in higher toxicity rather than 1:9 combination against mosquito larvae with LC_{50} of 24 and 32 mg/l, respectively[23]. In India, a study found that PE extract of tuba root also resulted in higher toxicity against *Ae. aegypti* larvae rather than the combination of the methanol-chloroform extract with LC_{50} of 0.616 mg/l and 4.21 mg/l, respectively[24]. Similar studies have also been reported from Indonesia. A study on toxicity of liquid ethanolic extract of tuba root against the filial one (F1) larvae of wild-caught *Ae. aegypti* larvae showed that the concentration 0.5% caused 86% of mortality rate[25], while another study using the ethanolic extract of tuba root showed the higher larvicidal potency against the laboratory strain of *Ae. aegypti* larvae with LC_{50} of 47.7526 mg/l[26]. But the temephos-resistant *Ae. aegypti* larvae needed a higher effective concentration of methanolic extract of tuba root with the LC_{50} and LC_{90} were 1,600 and 2,040 ppm, respectively[27]. These studies indicated that *D. elliptica* root extract has a variation of toxicity based on the extraction of solvents and habitat geographic origin. Another study showed that the number and type of secondary metabolites were influenced by the extraction solvent[28], while the composition of chemical constituents is affected by environmental habitat and climate conditions[29-32]. Based on the phenomenon, this in-vitro study aims to obtain the highest toxicity, and the effective concentration of the local *D. elliptica* extract against the 3rd-instar larvae of

laboratory strain *Ae*. *aegypti* based on the distilled water, methanol, ethyl acetate, and n-hexane extract types.

Materials and Methods

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Plant origin, collection, and preparation of extract

Tuba roots were taken from uncultivated lands in the hilly areas of the Samping village of Kemiri sub-district of Purworejo district, Central Java Province, Indonesia. The vine stems of plants in the ground were gently pulled out so that the roots did not break. The base of roots was cut, cleaned, and dried in the shade, before being sent to the laboratory for the extraction process. ~~We~~ Study used the previous procedure of extraction and fractionation[33-35] with modification (**Fig.1**). Briefly, the crude extract was obtained by maceration of six kilograms of tuba root dry powder in methanol for 3x24 hours. The filtrate was separated from the residue and evaporated to produces 400 g of methanol extract. The polarity of the extract was separated by the liquid-liquid partition method. As much as 250 ml of aqua dest was added to 120 g of solid methanol extract and stirred until completely homogeneous. Homogenate was entered into a 500 ml separation funnel, and 250 ml of n-hexane was added to separate the low polarity compounds, then shaken until it completely separates the top layer (n-hexane phase) and the bottom (water phase). The top and bottom layers were separated. The top layer was evaporated to obtain the solid n-hexane extract. In the lower part, ethyl acetate was added to separate the semi-polar compounds. The mixture was processed with a separation funnel like the previous procedure to obtain the upper layer (ethyl-acetate phase) and the lower (water layer). Both fractions were

evaporated separately to obtain the solid ethyl acetic and water extracts. A part of the four types of extracts were prepared for larvicidal activity (bioassay) test and phytochemical analysis using the Fourier Transform Infrared (FTIR) spectrophotometer.

Experimental mosquitoes

The parental *Ae. aegypti* mosquitoes were obtained in the larval stage from Sendang Mulyo village of Blora district, Central Java Province, Indonesia. Larvae were maintained to be the adult mosquito in the Laboratory of Epidemiology and Tropical Diseases, Public Health Faculty, Universitas Muhammadiyah Semarang. Species determination used the Walter Reed identification keys[36]. To obtain thousands of larvae with the same age, the parental *Ae. aegypti* mosquito was reared up to the third generation. During the rearing process, mosquitoes were fed with guinea pig's blood, and larvae were fed with dog food. The experiment temperature condition was maintained at the range of 25-28^{0}C and humidity of 70-80%. The late third or early of the fourth instar of filial (F3) larvae was subjected to the bioassay test[37].

Larvicidal bioassay

To determine the larvicidal toxicity of the *D. elliptica* root extracts, the WHO guideline was used[34]. Briefly based on the modification of previous study[24], the initial bioassay used four concentration ranges, namely 50, 100, 500, and 1000 mg/l in 100 ml distilled water for each *D. elliptica* root extract from the four solvents (methanol, n-hexane, ethyl acetate, and distilled water), and placed in plastic cup. Each concentration level was prepared five times replication so that there were total of 20 cups in each group of extract type. A total of 25 third instar larvae of *Ae. aegypti* were contacted for 24 hours with the *D. elliptica* root extract solution in each cup.

Two control groups were provided in this experiment, namely 0.02 mg/l temephos solution as the positive control, and distilled water as the negative control. Knockdown larvae of each container were observed in 30, 60, 120, 240, 480, and 1,440-minutes experiments. Larval mortality in each container was calculated after 24 hours of observation. The temperature was maintained at 25-28^{0}C. The initial bioassay showed that the concentration up to 100 ppm causes a range of 96-100% of the mortality rate of *Ae. aegypti* larvae among methanol, n-hexane, and ethyl acetate extract types. There were not dead mosquito larvae found in the distilled water extract so that the next step of the bioassay test for this extract type was stopped. Based on the results, it set a new concentration range of 3, 5, 10, 25, 50, and 100 mg/l for the three extract types, namely methanol, n-hexane, and ethyl-acetate.

Statistical analysis

The data of this study are presented descriptively in minimum – maximum, mean ± standard deviation (SD), mean and 95% confidence interval (CI), and analytically in compare mean by using two-way analysis of variance (ANOVA). LC_{50} and LC_{90} were determined by using the probit analysis. All the data analysis was performed by the SPSS statistical software. ~~The results of the data analysis were presented in tables and figures.~~

Ethical Approval

Ethics approval of this study was obtained from the Ethics Committee of Health Research of Public Health Faculty of Universitas Muhammadiyah Semarang with registration number 231/KEPK-FKM/UNIMUS/2019.

Results

Extract types and phytochemical compounds

Four extract types, namely methanol, n-hexane, ethyl acetate, and distilled water representing the polar, non-polar, semi-polar, and high polar extract (**Fig.1**) were obtained. Overall, the results of the FTIR analysis indicated nine phytochemical compounds that were distributed to four types of extracts. The findings show that each type of extract can contain several phytochemical compounds that are also found in other types of extracts, although the only rotenone and ceramides were found in all extract types (**Table 1**). The results indicated that the semi and non-polar solvents can bind more groups of phytochemical compounds.

Initial bioassay test

Based on the experimented concentration ranges of tuba root extract, there were three extract types showed the high toxicity against the 3rd instar larvae of *Ae. aegypti*, except the water extract. Twenty-four-hours exposure of the water extract has not been caused the larval mortality among all of the concentration ranges so that this extract type was excluded from the next experiment. Exposure of the lowest concentration (50 mg/l) of the n-hexane, methanol, and ethyl acetic extract has resulted in the larval mortality rate 100%, 98.4%, and 86% respectively. One hundred percent of the larval mortality rate was reached by the concentration of 100 mg/l among the three extract types.

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Bioassay test with the specific concentration ranges

The six concentration ranges of *D. elliptica* root extract tested shows the larval mortality rate of the 3rd instar larvae of *Ae. aegypti* have been found since the lowest concentration (3 mg/l), and

increase directly proportional to the concentration. The range of larval mortality in each extract type was 18.4-100%, 1.6-99.2%, and 0.8-98.4% for n-hexane, methanol, and ethyl- acetate, respectively. Average larval mortality of 100% was only found in the n-hexane extract, even since the concentration was 25 ppm (**Table 2**). This finding indicated that the n-hexane extract has a higher and faster larvicidal activity rather than the others.

Larvicidal activity of the three types of *D. elliptica* root extract also indicated a high effectiveness level, which was shown by LC_{50} and LC_{90} of the probit analysis results of 4,088 and 6,709 mg/l, 14,066 and 35,237 mg/l, and 21,063 and 60,096 mg/l for n-hexane, methanol, and ethyl-acetate, respectively (**Table 3**). Overall, the result of the two-way ANOVA test showed the differences of larvicidal activity of the *D. elliptica* root against the 3rd instar larvae of *Aedes aegypti* based on the interaction of the extract types and concentration levels (**Fig.2**). Pairwise comparisons of the larval mortality rate showed significant differences between the extract types (**Table 4**).

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In detail, the differences of larvicidal activity of *D. elliptica* root extract were shown by the knockdown larvae in each extract type based on the concentration and exposure time (**Fig.3**). The n-hexane extract showed the highest and fastest larvicidal activity since the lowest concentration and early exposure time. The different condition was shown by the methanol and ethyl-acetate extracts, where the significantly increasing of larvicidal activity was started by the concentration of 25 mg/l, and progressively increase in the higher concentrations. However, an average of 100% larval mortality rate was not reached by the methanol and ethyl-acetate extract types.

Discussion

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The history of research on *D. elliptica* in the context of biochemical compounds has gained various findings in the last century, since the use of freshly squeezed as the fish poisons and plant pests[11,12] and initial exploration of various secondary metabolites[18-20] to the isolation of specific chemical compounds in the last decade[13-17]. However, *D. elliptica* still has an interesting and promising potency to be researched and generate new findings. In the context of exploration and testing of the larvicidal activity of *D. elliptica* extracts against *Ae. aegypti* larvae in the last two decades at least six solvents have been used by researchers, namely petroleum ether, methanol, ethanol, and a combination of methanol: chloroform and methyl-chloride: methanol[22-26]. The bioassay test of the larvicidal activity of these extract types showed various results. Petroleum ether extract provides a different effect based on the geographical origin of plant habitat where in Thailand shows LC_{50} of 11.17 mg/l, whereas in India 0.616 mg/l. The larvicide toxicity test of methanol extract was carried out in Thailand and Indonesia (in this study) with equivalent results respectively 13.17 and 14.066 mg/l. This phenomenon is due to differences in levels of secondary metabolites of *D. elliptica* among regions[24,29-32]. The toxicity of plant extract from a combination of methanol-chloroform solvent was more effective than methyl chloride-methanol with LC_{50} of 4.21 and 24-32 mg/l, respectively. This condition showed that the solvent types affected the dissolved secondary metabolites[28].

This research is part of the exploration of larvicide bioactive compounds from *D. elliptica* which has been carried out in the last century with variations in yield according to solvent extraction, habitat conditions, and geographical regions. The use of the sequential extraction and fractionation method[33-35] which was modified and guided by bioassay test successively produced crude extract methanol, and its derived-extracts from n-hexane (non-polar), ethyl acetate (semi-

polar), and water (polar) fractions. Each extract type was subjected to a bioassay test and showed different toxicity against *Ae. aegypti* larvae. This strategy was carried out to obtain the maximum fraction and type of extract from limited raw material[35] so it was more efficient when compared to the parallel method. Extraction in parallel with different solvents shows variations in the percentage of extract weight. Water solvents produce the highest proportion of extracts compared to ethyl acetate, ethanol, and hexane[38,39], while other findings show that methanol produces a greater proportion than ethanol and water[40] so that we used the methanol in the initial extraction.

Overall, the results of the bioassay test showed that three extract types of *D. elliptica* are effective compounds because they had high toxicity against *Ae. aegypti* larvae. Previous studies categorized the effectiveness of plant extract larvicides into four levels, namely less effective (LC_{50}>750 mg/l), effective (LC_{50}=100-750 mg/l), moderate larvicidal activity (LC_{50}=50-100 mg/l) and high (LC_{50}<50 mg/l)[22]. In this study, three types of extracts namely n-hexane, methanol, and ethyl acetate have high larvicidal toxicity, so that further research and development become a technical grade of larvicide are still underway. The mortality rate of *Aedes aegypti* larvae in the bioassay test was caused by the exposure of *D. elliptica* root extracts. It is proven that there are no dead larvae in the negative control (aqua dest) and 100% of larvae die in positive control. Based on the WHO standard procedure, if larval mortality in the control group was less than 20%, the results of the bioassay test can still be accepted after being corrected with the Abbott formula[37].

The n-hexane extract type causes the highest *Ae. aegypti* larvae compared with the other extract types since the beginning of the exposure time and progressively continues to increase for up to 24 hours. The 97.6% mortality rate of *Ae. aegypti* larvae were achieved at a concentration

of 10 mg/l. The toxicity of n-hexane extract is related to secondary lipophilic metabolites contained in this extract type[39]. Phytochemical screening shows that there are nine secondary metabolites found in n-hexane extract types. The n-hexane extract has never been used in the previous study in the context of the larvicidal activity test of *D. elliptica* against *Ae. aegypti* larvae. Although the toxicity of n-hexane extract is lower than petroleum ether extracts[24], this solvent has resulted in a promising extract. Larvicidal toxicity test of ethyl acetate extract of *D. elliptica* root against *Ae. aegypti* larvae have not been performed yet. A study explored several classes of chemical compounds from *D. elliptica*, which are bound by ethyl acetate solvents namely alkaloids, flavonoids, sterols, tannins, and triterpenoids[41], and tested for antimicrobial activity. The FTIR analysis of this study indicated the same lead compounds between n-hexane extract and ethyl acetate.

The methanol extract in this study showed high larvicidal toxicity with LC_{50} which was almost equivalent to previous findings in Thailand[22], but lower than findings in India[24]. This solvent is polar and it was used as an initial extraction so that it can bind many classes of chemical compounds to a broad polarity spectrum[35,42]. Phytochemical screening results indicate six classes of chemical compounds contained in the methanol extract.

FTIR results show that the most important differences in the classes of lead compounds in the three types of extracts are stilbenes, isoflavones, and polyhydroxy acids. The toxicity of stilbenoids to mosquito larvae is determined by its lipophilic level[43], whereas lipophilic compounds are bound by hexane solvents. This result indicates that stilbenoids are important chemical compounds in n-hexane extract.

Water extracts have not indicated the larvicidal toxicity against the 3rd instar larvae of *Ae. aegypti* up to the concentration of 1,000 mg/l, whereas the other extract types have killed 100%

of larvae at the concentration of 100 mg/l. The possible reasons are the complexity or low levels of chemical compounds bound by this universal solvent. If the bound compounds are very complex and contain many types of chemical compounds, there is a possibility of an antagonistic mechanism between the compounds[44,45]. The second possible reason is that the water extract resulted from the last fraction so that the extract contains only a few remaining compounds, both types, and levels. Although water is the universal extraction solvent, this extract only contains the polar chemical compounds bound to methanol and can be bound by water, at a low level[46]. Both of these conditions are still unclear and interesting for further study.

Exploration of various bioactive larvicidal ingredients from the roots of *D. elliptica* is an important effort in the context of Dengue vector control considering that the temephos resistance of *Ae. aegypti* larvae are increasingly widespread and have been reported in various endemic areas of Dengue[6-8]. Based on these conditions, community attention has increased on the natural insecticides and larvicides compounds because their advantages are easily decomposed and bioaccumulation does not occur in the environment[9]. Bioassay tests on extracts from several types of plants, including *D. Elliptica* have found promising results where the findings present the varied but low LC_{50}[22,24,47].

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Conclusion

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The sequential extraction and fractionation of *D. elliptica* root resulted in three of four fractions as the effective extract that the extract types have high larvicidal toxicity against the 3rd instar larvae of *Ae. aegypti*. The bioassay test result showed that n-hexane fraction has the highest toxicity and provides the lowest LC_{50} followed by methanol and ethyl acetate, while water extract has not indicated the larvicidal activity up to the highest tested concentration of 1,000

mg/l. The high toxicity of the n-hexane extract is related to the lipophilic compounds contained, and stilbene is thought to play a role in this case. There were nine lead compounds indicated from the extract types where rotenone and ceramides were found in each extract type. FTIR analysis indicated that n-hexane and ethyl acetate extract contain similar lead compounds. Three lead compounds, namely stilbenes, isoflavones, and polyhydroxy acid were not found in methanol and water extract. Further studies are necessary to be conducted to isolate and characterize the pure or specific compounds from the extract with the highest toxicity, followed by the formulation of the technical grade larvicide guided with bioassay test against the 3rd instar larvae of *Ae. aegypti* both in susceptible and temephos-resistant strains.

Conflict of Interest

Authors of this paper have no conflict of interest.

Authors' contributions

Sayono Sayono constructed the main idea of the study, supervise all of the research activities, thoroughly analyzed the data, and wrote the main contents of the manuscript. Risyandi Anwar carried out the phytochemical analysis and extraction of *D. elliptica* roots in the natural chemistry laboratory, and supported manuscript draft related to the extraction process and results. Didik Sumanto carried out the mosquito rearing and larvicidal bioassay and supported the manuscript draft about the bioassay test. All authors discussed the final manuscript.

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References

1. Kraemer, M.U., M.E. Sinka, K.A. Duda, A.Q.N. Mylne, and F.M. Shearer, et al, 2015. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. Albopictus*. *Elife*, 4: e08347. doi: 10.7554/eLife.08347.
2. WHO, 2019. Dengue and Severe Dengue. Geneva, Switzerland. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue. Accessed on September 15, 2019.
3. Pérez, D., P. Lefèvre, L. Sánchez, L.M. Sánchez, M. Boelaert, G. Kourí, P.V. der Stuyft, 2007. Community participation in *Aedes aegypti* control: a sociological perspective on five years of research in the health area ‘‘26 de Julio’’, Havana, Cuba. Trop. Med. Int. Health., 12(5):664–672. doi:10.1111/j.1365-3156.2007.01833.x.
4. Sayono, S., W. Widoyono, D. Sumanto, R. Rokhani, 2019. Impact of Dengue Surveillance Workers on Community Participation and Satisfaction of Dengue Virus Control Measures in Semarang Municipality, Indonesia: A Policy Breakthrough in Public Health Action. Osong Public Health Res Perspect. 10(6):376-385. doi: 10.24171/j.phrp.2019.10.6.08

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5. George, L., A. Lenhart, J. Toledo, A. Lazaro, and W.W. Han, et al, Community-Effectiveness of Temephos for Dengue Vector Control: A Systematic Literature Review. PLoS Negl. Trop. Dis., 9(9): e0004006, 2015. doi:10.1371/journal.pn-td.0004006
6. Moyes, C., J. Vontas, A.J. Martins, L.C. Ng, S.Y. Koou, and I. Dufour, et al., 2017. Contemporary status of insecticides resistance in the major Aedes vectors of arbociruses infecting humans. PLoS Negl. Trop. Dis., 11(7):e0005625. Doi: 10.1371/journal.pntd.0005625
7. Mulyatno, K.C., A. Yamanaka, Ngadino, E. Konishi, 2012. Resistance of *Aedes aegypti* (Lin.) larvae to temephos in Surabaya, Indonesia. Southeast Asian J. Trop. Med. Public Health, 43(1):29–33. PMID: 23082551
8. Ikawati, B., Sunaryo, Wahyudi, B. Fajar, 2017. *Aedes aegypti* Resistance to Temephos in Central Java, Indonesia. Adv. Sci. Lett., 23(4):3544–3546. DOI**:** https://doi.org/10.1166/asl.2017.9163
9. Arnason, J.T., S.R. Sims, I.M. Scott, 2012. Natural products from plants as insecticides. In: Phytoc-hemistry and Pharmacognosy. Encyclopedia of Life Support System (EoLSS). Available from: https://www.eolss.net/Sample-Chapters/C06/E6-151-13.pdf
10. Starr, F., K. Starr, L. Loope, 2003. *Derris elliptica*: *poison vine, fabaceae*; 1-4. Available from: http://www.hear.org/starr/hiplants/reports/pdf/derris_elliptica.pdf
11. Orwa, C., A. Mutua, R. Kindt, R. Jamnadass, S. Anthony, 2009. Agroforestree Database: a tree reference and selection guide version 4.0. available from: http://www.worldagroforestry.org/sit-es/treedbs/treedatabases.asp)
12. Sirichamorn, Y., F.A.C.B. Adema, B. Gravendeel, P.C. Welzen, 2012. Phylogeny of palaeotropic *Derris*-like taxa (Fabaceae) based on chloroplast and nuclear DNA sequences

shows reorganization of (infra) generic classifications is needed. Am. J. Bot., 99(11):1793–1808. http://doi.org/10.3732/ajb.1200390.

13. Morel, S., J.J. Helesbeux, D. Séraphin, S. Derbré, J. Gato, and M-C. Aumond, et al., 2013. Anti-AGEs and antiparasitic activity of an original prenylated isoflavonoid and flavonone isolated from *Derris ferruginea*. Phytochem. Lett., 6:498–503. http://dx.doi.org/10.1016/j.phytol.2-013.06.002.
14. Wu, X., Z. Song, H. Xu, H. Zhang, and W. Chen, et al., 2012. Chemical constituents of the aerial part of *Derris elliptica*. Fitoterapia, vol. 83, no. 4, pp. 723–736, 2012. doi:10.1016/j.fitote.2012.02.2015.
15. Musa, W., J. Ahmad, C.J. Lamangantjo, 2016. Bioactive Compounds in Tombili Seeds and Tubile Roots as the Alternative for Synthetic Pesticide to Protect Wheats from Insects and Pests. Int. J. ChemTech Res., 9(4):604–615. http://www.sphinxsai.com/2016/ch_vol9_n-o4/3/(604-616)V9N4CT.pdf
16. Lu, H.Y, 2008. Isoflavones and their derivatives from the root of *Derris elliptica* (Roxb.) Benth. Chin. J. Nat. Medicines. 6(4):265–267. DOI:10.3724/ SP.J.1009.2008.00265
17. Russel, D.A., W.J.S. Fong, G.G. Twigg, H.F. Sore, and D.R. Spring, 2017. Stereocontrolled semisyntheses of elliptone and 12aβ-Hydroxyelliptone. J. Nat. Prod., 80(10):2751-2755. https://doi.org-/10.1021/acs.jnatprod.7b00527
18. Seiferle, E.J., D.E.H. Frear, 1948. Insecticides derived from plants. Ind. Eng. Chem., 40(4):683–691. https://doi.org/10.1021/ie50460a022
19. Gupta, R.C., 2007. Rotenone, in Veterinary Toxicology: Basic and Clinical Principles, Academic Press.

20. Yoon, A.S., 2009. Extraction and Formulation Development of Derris elliptica for Insect Pest Control. [Ph.D Thesis]. Prince of Songkla University, Thailand. http://kb.psu.ac.th/psukb/bitstream/2010/7072/1/316615.pdf

21. Zubairi, S.I., M.R. Sarmidi, R.A. Aziz, 2014. Bio-Active Constituents of Rotenoids Resin Extracted from Derris elliptica Roots: Comparison between Local Plant Extract and SAPHYR (France) Cube Resin. Adv. Environ. Biol., 8(4):904–909

22. Komalamisra, N., Y. Trongtokit, R. Rongsriyam, and C. Apiwathnasorn, 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.

23. Zubairi, S.I., M.R. Sarmidi, and R.A. Aziz, 2015. A preliminary study on mosquito larvicidal efficacy of rotenone extracted from Malaysia Derris sp. J. Teknol., 76(1):275–279. Available from www.jurnalteknologi.utm.my

24. Dohutia, C., D.R. Bhattacharyya, S.K. Sharma, P.K. Mohapatra, and K. Gogoi, et al., 2015. Larvicidal activity of few select indigenous plants of North East India against disease vector mosquitoes (Diptera: Culicidae. Trop. Biomed., 32(1):17–23. Available: https://www.ncbi.nlm.nih.gov/pubmed/25801251

25. Sayono, U. Nurullita, M. Suryani, 2010. Pengaruh konsentrasi flavonoid dalam ekstrak akar tuba (*Derris elliptica*) terhadap kematian larva Aedes aegypti. J. Kesehat. Masy. Indones., 6(1):38-47.

26. Komansilan, A., N.W. Suriani, H.T. Lawalata, 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

27. Sayono, S., A. Permatasari, D. Sumanto, 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.
28. Nguyen, A., Y. Phan, 2014. Preliminary phytochemical analysis of different solvent extracts of Derris elliptica (Roxb.) Benth leaves. International Journal of innovation and Applied Research, 2(12):74–76. Available from http://www.journalijiar.com.
29. Karahan, F., C. Avsar, I.I. Ozyigit, et al., 2016. Antimicrobial and antioxidant activities of med-icinal plant *Glycyrrhiza glabra* var: *gladulifera* from different habitats. Biotechnol. Biotech. Eq., 30(4):797–804. https://doi.org/10.1080/ 13102818.2016.1179590
30. Kumar, S., A. Yadav, M. Yadav, and J.P. Yadav, 2017. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm.f. BMC Res. Notes, 10(60). DOI 10.1186/s13104-017-2385-3
31. Sampio, B.L., F.B. Da-Costa, 2018. Influence of abiotic environmental factors on the main cons-tituents of the volatile oils of *Tithonia diversifolia*. Rev. Bras. Farmacogn., 28(2):135–144. https://doi.org/10.1016/j.bjp.2018.02.005
32. Qi, M., X. Hua, X. Peng, X. Yan, and J. Lin, 2018. Comparison of chemical composition in the buds of *Aralia elata* from different geographical origins of China. R. Soc. Open Sci., 5:180676. http://dx.doi.org/10.1098/rsos/180676.
33. Li, J.X., T. Hareyama, Y. Tezuka. Y. Zhang, T. Miyagara, and S. Kadota, 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

34. Koehn, F.E., G T. Carter, 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov., 4(3):206–220. doi:10.1038/nrd1657
35. Liu, Z., 2008. Preparation of botanical samples for biomedical research. Endocr. Metab. Immune Disord. Drug Targets, 8(2):112–121. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936020/pdf/nihms556279.pdf
36. Walter Reed Biosystematics Unit. Mosquito identification keys. http://www.wrbu.org/aors/pacom_Keys.html
37. WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides, Geneva, Switzerland:7-12. Available from https://apps.who.int/iris/h-andle/10665/69101
38. Razak, M.F.B.A., P.K. Yong, Z.M. Shah, L.C. Abdullah, S.S. Yee, and I.T.C.S. Yaw, 2012. The effect of varying solvent polarity on extraction yield of *Orthosiphon stamineus* leaves. J. Appl. Sci., 12(11):1207–1210.
39. Abarca-Vargas, R., C.F.P. Malacara, V.L. Petricevich, 2016. Characterization of Chemical Compounds with Antioxidant and Cytotoxic Activities in Bougainvillea x buttiana Hol-ttum and Standl, (var. Rose) Extracts. Antioxidants, 5(45):1-11. doi:10.3390/anti-ox5040045. www.mdpi.com/journal/antioxidants.
40. Borah, R., S.P. Biwas, 2018. Tulsi (Ocimum sanctum), excellent source of phytochemicals. Int. J. Environ. Agric. Biotech., 3(5):1732-1738. http://dx.doi.org/10.22161/ijeab/-3.5.21
41. Khan, M.R., A.D. Omoloso, Y. Barewai, 2006. Antimicrobial activity of the Derris elliptica, Derris indica and Derris trifoliata extractives. Fitoterapia, 77(4):327-330. doi:10.1016/j.-fitote.2006.03.007

42. Tiwari, P., B. Kumar, M. Kaur, and H. Kaur, 2011. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia, vol. 1, no. 1, pp. 98-106, 2011. Available online from http://www.ipharmsciencia.com

43. Sobolev, V.S., S.I. Khan, N. Tabanca et al., 2011. Biological activity of peanut (Arachis hypogaea) phytoalexins and selected natural and synthetic stilbenoids. J. Agric. Food Chem., 59(5):1673-1682. doi:10.1021/jf104742n

44. Milugo, T.K., L.K. Omosa, O.J. Ochanda, B.O. Owuor, and F.A. Wamunyokoli, et al., 2013. Antagonistic effect of alkaloids andsaponins on bioactivity in the quinine tree (Rauv-olfia caffrasond.): further evidence to support biotechnology in traditional medicinal plants. BMC Complement. Altern. Med., 13(285):285. doi:10.1186/1472-6882-13-285

45. Celep, E., Y. İnan, S. Akyüz, and E. Yesilada, 2017. The bioaccessible phenolic profile and antioxidant potential of Hypericum perfoliatum L. after simulated human digestion. Ind. Crops Prod., 109:717-723. http://dx.doi.org/10.1016/j.indcrop.2017.09.032

46. Dawan, D., J. Gupta, 2017. Comparison of Different Solvents for Phytochemical Extraction Potential from Datura metel Plant Leaves. Int. J. Biol. Chem., 11(1)::17-22. doi:10.3923/i-jbc.2017.17.22

47. Scalvenzi, L., M. Radice, L. Toma, F. Severini, and D. Boccolini, et al., 2019. Larvicidal activity of *Ocimum campechianum*, *Ocotea quixos* and *Piper aduncum* essential oils against *Aedes aegypti*. Parasite, 26(23):1-8. https://doi.org/10.1051/parasite/2019024.

Table 1. FTIR analysis of four extract types of Derris elliptica root indicates nine phytochemical compounds.

Lead compounds	Extract types			
	Methanol	n-Hexane	Ethyl acetate	Distilled-water
Rotenone	+	+	+	+
Pterocarpans	+	+	+	-
Cumestans	+	+	+	-
Flavone	+	+	+	-
Anthraquinone	+	+	+	-
Ceramides	+	+	+	+
Stilbenes	-	+	+	-
Isoflavones	-	+	+	+
Poly-hydroxy acids	-	+	+	+

+ = Present and - = Absent

Table 2. Mortality rate of the 3rd instar larvae of *Aedes aegypti* based on the extract types and concentrations of the *Derris elliptica* root extract

Extract types	**Concentration (mg/l)**	**Minimum**	**Maximum**	**Mean**	**Std. deviation**
Methanol	3	0	4	1.6	2.19
	5	4	24	12.8	9.12
	10	12	32	21.6	7.79
	25	68	92	80.0	8.94
	50	96	100	98.4	2.19
	100	96	100	99.2	1.79
Ethyl acetate	3	0	4	1.6	2.19
	5	4	8	4.8	1.79
	10	12	24	16.8	4.38
	25	48	64	54.4	6.07
	50	80	92	86.4	4.56
	100	96	100	98.4	2.19
n-Hexane	3	4	32	18.4	12.84
	5	52	88	75.2	13.98
	10	96	100	97.6	2.19
	25	100	100	100	0.00
	50	100	100	100	0.00
	100	100	100	100	0.00
Negative control (-)	-	0	0	0	0
Positive control (+)	0.02	100	100	100	0

(-) aquadest; (+) temephos 0.02 mg/l

1

Table 3. Results of Probit analysis showed the LC_{50} and LC_{90} of the extract toxicity of *Derris elliptica* root against the 3rd instar larvae of *Aedes aegypti*

Extract types	Regression equation	Lethal Concentration (mg/l)		Chi Square	p
		LC50 (95% confidence limits)	LC90 (95% confidence limits)		
Methanol	Y= -3.689+3.213X	14.066 (10.700 – 18.755)	35.237 (25.217 – 61.023)	15.004	0.005
Ethyl acetate	Y= -3.725+2.815X	21.063 (18.987 – 23.389)	60.096 (51.814 – 71.695)	2.764	0.598
n-Hexane	Y= -3.637+5.950X	4.086 (3.825 – 4.355)	6.709 (6.144 – 7.603)	4.530	0.339

Table 4. Pairwise comparisons of larval mortality based on the extract types

Pairwised extract types	Mean difference	p	95% confidence interval for difference
Methanol >< Ethyl acetate	8.533	0.035	0.611 – 16.455
n-Hexane >< Ethyl acetate	38.133	0.000	30.211 – 46.055
n-Hexane >< Methanol	29.600	0.000	21.678 – 37.522

Figures

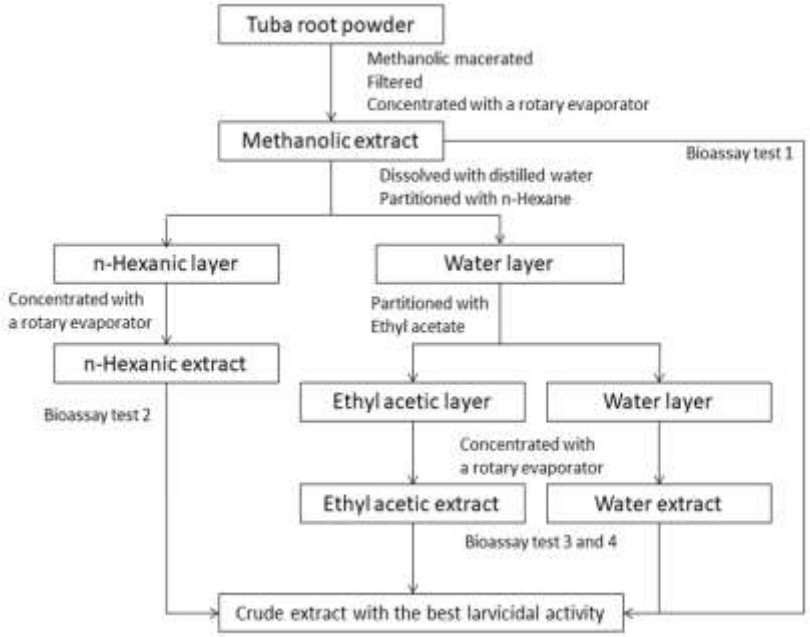

Fig.1. The extraction and sequential fractionation of *Derris elliptica* root using four different polarity solvents. Crude extract was obtained from methanol-macerated of tuba root powder, filtered, and evaporated by using rotary evaporator. A part of crude extract was dissolved in distilled-water and fractionated with n-hexane to separate the n-hexane and water layer. The water layer was fractionated with ethyl acetate resulted the ethyl acetate and water layer. All fractions (n-hexane, ethyl acetate, and water layers) were evaporated to result three extract types. The four of extract types were subjected into bioassay test for determining the toxicity against the 3rd instar larvae of *Aedes aegypti*.

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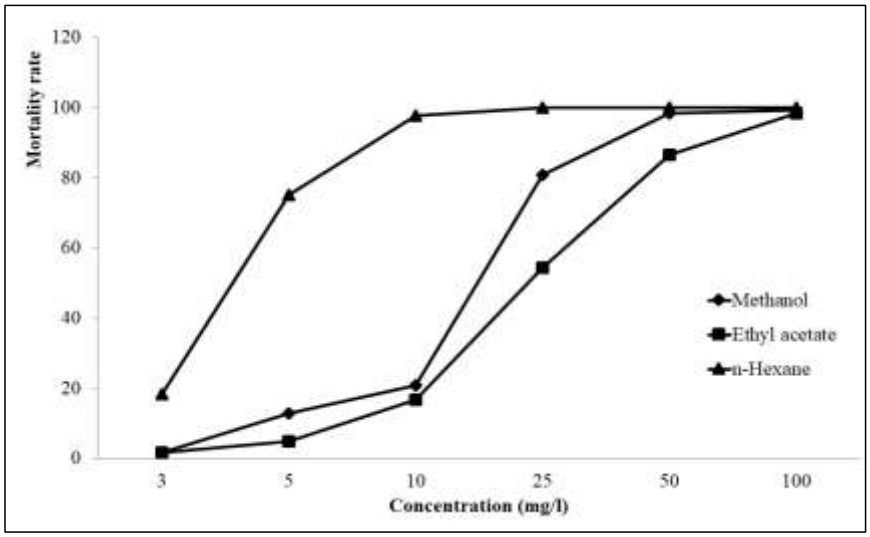

Fig.2. Results of ANOVA test showed the interaction effects of the extract types and concentrations of tuba root extract to the mortality rate of the 3rd instar larvae of *Aedes aegypti*. The highest mortality rate was observed in n-hexane extract.

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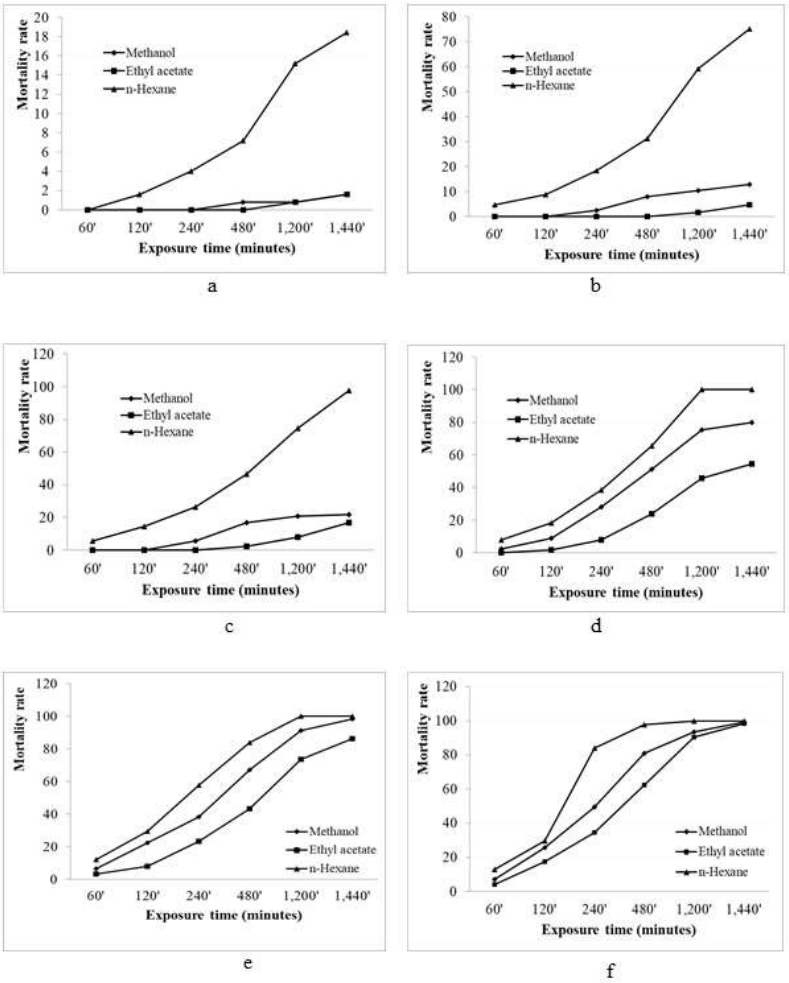

Fig.3. Results of bioassay test with specific concentration ranges showed the different of larvicidal activity based on the extract types, exposure times, and concentrations. Details of larvicidal activity are showed in the letter bellow: a=3 mg/l, b=5 mg/l, c=10 mg/l, d=25 mg/l, e=50 mg/l, and f=100 mg/l. The n-hexanic extract caused the higher and faster of knockdown effect since the lowest concentration rather than the others. Increasing of larvicidal activities of methanol and ethyl-acetate extract were significantly started in concentration of 25 mg/l although need longer exposure times.

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You can explain figure with detail explanation in main Results section.

Evaluation Report

Final Decision: Reconsider for Evaluation after Modifications and Clarifications

Article No.: 102970-PJBS-ANSI **Article Type: Research article**
Tables Available: 4 Tables Cited: 4
Figures Available: 3 Figures Cited: 3
Manuscript falls in the scope of the journal? Yes

My observations/comments about this article are:

No.	Part	Comments	Author Response
1.	Cover letter	• Overall OK	
2.	Write up	• Overall OK	
3.	Title	• Overall OK	
4.	Running Title	• Running title of the study is missing. Provide a running title of 5-7 words besides the main title in manuscript and it must be the punch line of main title	We have added the running title as suggested (in yellow high light)
5.	Author's Information	• Overall OK	
6.	Author's Contribution	• Overall OK	
7.	Abstract	• Overall OK	
8.	Keywords	• Overall OK	
9.	Introduction	• Overall OK	
10.	Materials and Methods	• When the study was carried out? Add time duration (in specific	We have added time duration for conducting research, and subheading as

		months and years) of your research work in the start of "Materials and Methods" under the subheading "Study area"	suggested (in yellow high light)
11.	**Results**	• Author should explain **"Initial Bioassay Test"** in tabulated or graphical form for better understanding.	We have added one table (**Table 1**) to explain the results of the **Initial bioassay test**. This insert causes the changes of order number of the other tables, and we have created a new sequence (in yellow high light).
		• Provide more explanation of Table 4 in text for better understanding of findings.	We have added additional explanations for **Table 4** (now **Table 5**) as suggested (in yellow high light).
12.	**Figures**	• Author should provide clear cut caption of figure which explain figure 1 and 2 self-explanatory. Caption should be attractive, simple and precise. Author should extract the main caption of the figure 1 and rest of the text should be added under the citation of figure 1 in Results section • Author should present the	We have simplified the caption of all **Figures** as suggested (in yellow high light).

		caption of each part of the figure 3 separately for clear understanding. Avoid over explanation of data in caption. • You can explain figure with detail explanation in main Results section.	
13.	**Tables**	• Overall OK	
14.	**Discussion**	• Author should avoid over explanation of data in Discussion section. Just co-relate your findings with previous studies in favor as well as in contradiction. • In the last give limitations and suggestion about your study.	
15.	**Conclusion**	• This section should be precise in 5-6 lines only. Author should give his final remarks only in this section.	
16.	**Acknowledgement**	• Overall OK	
17.	**Significance Statement**	• Please write the Significance statement as follows: “This study	We have added a subheading "Significance statement" and relevant information

		discovered the ---- that can be beneficial for ---- and last sentence of this statement could be as: this study will help the researchers to uncover the critical areas of ---- that many researchers were not able to explore. Thus a new theory on ----- may be arrived at"	according to the reviewer's instructions
18.	**References**	• Author did not provide the DOI of 4 references in Reference section (Highlighted). Provide correct and active DOI/URL.	We have added DOI/URL for the four references

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- Author is requested to please highlight the amended portion in the manuscript. It will be more helpful for us in cross checking of suggested modifications.
- Please give your response in the Evaluation report as well under the column **"Author Response"** for all the parts of the manuscript.

Incorporate all the recommended modifications

~~Toxicity~~ Evaluation of Toxicity in Four Extract Types of Tuba Root against Dengue Vector, *Aedes aegypti* [Diptera:Culicidae] Larvae: an Invitro Study on Larvicidal Potency

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Abstract

Background and Objectives: Since the Dengue virus spreads rapidly and the vector becomes resistant to insecticides and larvicides, exploration of new compounds which overcome resistance problems, are easily degraded, and do not lead to bioaccumulation, is needed. This study evaluated four extract types of *Derris elliptica* represented the polar, semi-polar, and nonpolar extract against the 3rd-instar larvae of *Ae. aegypti*, and determined the effective concentration among the extracts. **Material and Methods:** The crude extract was obtained from the maceration of root powder of the plant with methanol, and subsequently evaporated. The crude extract was diluted in distilled water and partitioned sequentially with ethyl-acetate, n-hexane, and water to obtain their fractions. All the fractions were evaporated to obtain their extract types. Initial bioassay test of the extracts with concentration ranges of 50, 100, 500, and 1,000 mg/l against *Ae. aegypti* larvae ~~were done according to WHO procedure,~~ and resulted in 86-100% larval mortality rates at concentrations of 50 and 100 mg/l, except for water extract. The lower concentration range of 3, 5, 10, 25, 50, and 100 mg/l of three extract types were tested. **Results:** Larval mortality rates of 18.4–100%, 1.6–99.2%, and 0.8–98.4% with LC_{50} of 4.088, 14.066, and 21.063 mg/l respectively for n-hexane, methanol, and ethyl-acetate. FTIR analysis indicated nine lead compounds in which rotenone and ceramides were observed in all extract types. **Conclusion:** The n-hexane extract showed the highest larvicidal toxicity, and its specific compounds are necessarily isolated to obtain pure bioactive ingredients.

Keywords: larvicidal potency, *Derris elliptica*, *Aedes aegypti*, tuba root extract, n-hexane

Introduction

Since the Dengue virus spread rapidly in the past five decades from nine countries in 1970 to 128 countries in the tropics and subtropics recently[1,2], community attention and involvement in Dengue endemic areas in controlling *Aedes aegypti* mosquitoes has increased[3,4]. Aedes mosquito larvae become a strategic target in the Dengue vector control, where temephos rely upon larvicides. The campaign to use temephos is done seriously following and combining with the other methods to control the adult and larval stage of Aedes mosquitoes. This phenomenon occurs widely and intensively in endemic Dengue areas throughout the world for a long time[5] and results in the emergence of resistant strains of *Ae. aegypti* larvae against temephos, which have been reported in many countries[6], including in Indonesia[7,8].

The development of *Aedes aegypti* larvae resistance to temephos has hampered the Dengue vector control program. This condition triggers ~~scientists~~ researcher to find the new chemical compounds that are effective, biodegradable, and do not cause bioaccumulation in environment[9]. In line with these efforts, the utilization of the potential for tubal root toxicity has evolved from traditional to modern methods in solving the problem of controlling dengue.

Tuba (*D. elliptica* (Wallich) Benth) is a poisonous vine that is easily found on uncultivated agricultural land. This plant grows in the South Asian, Southeast Asian and Hawaiian regions[10]. Traditionally, the tuba roots have long been used by residents of the regions as a fish poison and plant pest pesticide[11,12]. The use of tuba root is related to chemical compounds contained in the plant comprising isoflavonoids[13], flavonoids[14,15], ceramides and polyhydroxy acids[16], as well as rotenoids[17] which include compounds such as rotenone, deguelin, toxicarol, sumatrol, elliptone, and malaccol[18-21].

Previously, the studies on the larvicidal toxicity of tuba root extract against *Ae. aegypti* larvae rapidly develop in several regions to find the new active compound of larvicide. A study in Thailand found that tuba root extract with petroleum ether (PE) and methanol solvents showed a different toxicity, where the PE extract showed the lower lethal concentration of 50% of mortality (LC_{50}) and LC_{90} rather than the others, namely 11.17 and 27.74 mg/l[22]. Two other studies in two different countries tested the *D. elliptica* root extract that was resulted from a combination of two solvents. In Malaysia, a 1:1 combination of methyl-chloride and methanol results in higher toxicity rather than 1:9 combination against mosquito larvae with LC_{50} of 24 and 32 mg/l, respectively[23]. In India, a study found that PE extract of tuba root also resulted in higher toxicity against *Ae. aegypti* larvae rather than the combination of the methanol-chloroform extract with LC_{50} of 0.616 mg/l and 4.21 mg/l, respectively[24]. Similar studies have also been reported from Indonesia. A study on toxicity of liquid ethanolic extract of tuba root against the filial one (F1) larvae of wild-caught *Ae. aegypti* larvae showed that the concentration 0.5% caused 86% of mortality rate[25], while another study using the ethanolic extract of tuba root showed the higher larvicidal potency against the laboratory strain of *Ae. aegypti* larvae with LC_{50} of 47.7526 mg/l[26]. But the temephos-resistant *Ae. aegypti* larvae needed a higher effective concentration of methanolic extract of tuba root with the LC_{50} and LC_{90} were 1,600 and 2,040 ppm, respectively[27]. These studies indicated that *D. elliptica* root extract has a variation of toxicity based on the extraction of solvents and habitat geographic origin. Another study showed that the number and type of secondary metabolites were influenced by the extraction solvent[28], while the composition of chemical constituents is affected by environmental habitat and climate conditions[29-32]. Based on the phenomenon, this in-vitro study aims to obtain the highest toxicity, and the effective concentration of the local *D. elliptica* extract against the 3rd-instar larvae of

laboratory strain *Ae*. *aegypti* based on the distilled water, methanol, ethyl acetate, and n-hexane extract types.

Materials and Methods

Study area

This study was conducted in eight months from March to October 2019. Tuba roots were taken from uncultivated lands in the hilly areas of the Samping village of Kemiri sub-district of Purworejo district, Central Java Province, Indonesia. Extraction process was conducted in the Natural Chemical Laboratory of Sciences and Mathematics Faculty of Garut University, West Java Province while the mosquito rearing and bioassay tests were conducted in the Epidemiology and Tropical Diseases Laboratory of Public Health Faculty of Universitas Muhammadiyah Semarang, Indonesia.

Plant collection and extraction

The vine stems of Tuba plants in the ground were gently pulled out so that the roots did not break. The base of roots was cut, cleaned, and dried in the shade, before being sent to the laboratory for the extraction process. ~~We~~ Study used the previous procedure of extraction and fractionation[33-35] with modification (**Fig.1**). Briefly, the crude extract was obtained by maceration of six kilograms of tuba root dry powder in methanol for 3x24 hours. The filtrate was separated from the residue and evaporated to produces 400 g of methanol extract. The polarity of the extract was separated by the liquid-liquid partition method. As much as 250 ml of aqua dest was added to 120 g of solid methanol extract and stirred until completely homogeneous.

Homogenate was entered into a 500 ml separation funnel, and 250 ml of n-hexane was added to separate the low polarity compounds, then shaken until it completely separates the top layer (n-hexane phase) and the bottom (water phase). The top and bottom layers were separated. The top layer was evaporated to obtain the solid n-hexane extract. In the lower part, ethyl acetate was added to separate the semi-polar compounds. The mixture was processed with a separation funnel like the previous procedure to obtain the upper layer (ethyl-acetate phase) and the lower (water layer). Both fractions were evaporated separately to obtain the solid ethyl acetic and water extracts. A part of the four types of extracts were prepared for larvicidal activity (bioassay) test and phytochemical analysis using the Fourier Transform Infrared (FTIR) spectrophotometer.

Experimental mosquitoes

The parental *Ae. aegypti* mosquitoes were obtained in the larval stage from Sendang Mulyo village of Blora district, Central Java Province, Indonesia. Larvae were maintained to be the adult mosquito in the Epidemiology and Tropical Diseases Laboratory. Species determination used the Walter Reed identification keys[36]. To obtain thousands of larvae with the same age, the parental *Ae. aegypti* mosquito was reared up to the third generation. During the rearing process, mosquitoes were fed with guinea pig's blood, and larvae were fed with dog food. The experiment temperature condition was maintained at the range of 25-28^{0}C and humidity of 70-80%. The late third or early of the fourth instar of filial (F3) larvae was subjected to the bioassay test[37].

Larvicidal bioassay

To determine the larvicidal toxicity of the *D. elliptica* root extracts, the WHO guideline was used[34]. Briefly based on the modification of previous study[24], the initial bioassay used four

concentration ranges, namely 50, 100, 500, and 1000 mg/l in 100 ml distilled water for each *D. elliptica* root extract from the four solvents (methanol, n-hexane, ethyl acetate, and distilled water), and placed in plastic cup. Each concentration level was prepared five times replication so that there were total of 20 cups in each group of extract type. A total of 25 third instar larvae of *Ae. aegypti* were contacted for 24 hours with the *D. elliptica* root extract solution in each cup. Two control groups were provided in this experiment, namely 0.02 mg/l temephos solution as the positive control, and distilled water as the negative control. Knockdown larvae of each container were observed in 30, 60, 120, 240, 480, and 1,440-minutes experiments. Larval mortality in each container was calculated after 24 hours of observation. The temperature was maintained at 25-28^{0}C. The initial bioassay showed that the concentration up to 100 ppm causes a range of 96-100% of the mortality rate of *Ae. aegypti* larvae among methanol, n-hexane, and ethyl acetate extract types. There were not dead mosquito larvae found in the distilled water extract so that the next step of the bioassay test for this extract type was stopped. Based on the results, it set a new concentration range of 3, 5, 10, 25, 50, and 100 mg/l for the three extract types, namely methanol, n-hexane, and ethyl-acetate.

Statistical analysis

The data of this study are presented descriptively in minimum – maximum, mean ± standard deviation (SD), mean and 95% confidence interval (CI), and analytically in compare mean by using two-way analysis of variance (ANOVA). LC_{50} and LC_{90} were determined by using the probit analysis. All the data analysis was performed by the SPSS statistical software. ~~The results of the data analysis were presented in tables and figures.~~

Ethical Approval

Ethics approval of this study was obtained from the Ethics Committee of Health Research of Public Health Faculty of Universitas Muhammadiyah Semarang with registration number 231/KEPK-FKM/UNIMUS/2019.

Results

Extract types and phytochemical compounds

Four extract types, namely methanol, n-hexane, ethyl acetate, and distilled water representing the polar, non-polar, semi-polar, and high polar extract (**Fig.1**) were obtained. Overall, the results of the FTIR analysis indicated nine phytochemical compounds that were distributed to four types of extracts. The findings show that each type of extract can contain several phytochemical compounds that are also found in other types of extracts, although the only rotenone and ceramides were found in all extract types (**Table 2**). The results indicated that the semi and non-polar solvents can bind more groups of phytochemical compounds.

Initial bioassay test

Based on the experimented concentration ranges of tuba root extract, there were three extract types showed the high toxicity against the 3rd instar larvae of *Ae. aegypti*, except the water extract. Twenty-four-hours exposure of the water extract has not been caused the larval mortality among all of the concentration ranges so that this extract type was excluded from the next experiment. Exposure of the lowest concentration (50 mg/l) of the n-hexane, methanol, and ethyl acetic extract has resulted in the larval mortality rate 100%, 98.4%, and 86% respectively (**Table**

1). One hundred percent of the larval mortality rate was reached by the concentration of 100 mg/l among the three extract types.

Bioassay test with the specific concentration ranges

The six concentration ranges of *D. elliptica* root extract tested shows the larval mortality rate of the 3rd instar larvae of *Ae. aegypti* have been found since the lowest concentration (3 mg/l), and increase directly proportional to the concentration. The range of larval mortality in each extract type was 18.4-100%, 1.6-99.2%, and 0.8-98.4% for n-hexane, methanol, and ethyl- acetate, respectively. Average larval mortality of 100% was only found in the n-hexane extract, even since the concentration was 25 ppm (**Table 3**). This finding indicated that the n-hexane extract has a higher and faster larvicidal activity rather than the others.

Larvicidal activity of the three types of *D. elliptica* root extract also indicated a high effectiveness level, which was shown by LC_{50} and LC_{90} of the probit analysis results of 4,088 and 6,709 mg/l, 14,066 and 35,237 mg/l, and 21,063 and 60,096 mg/l for n-hexane, methanol, and ethyl-acetate, respectively (**Table 4**). Overall, the result of the two-way ANOVA test showed the differences of larvicidal activity of the *D. elliptica* root against the 3rd instar larvae of *Aedes aegypti* based on the interaction of the extract types and concentration levels (**Fig.2**). Pairwise comparisons of the larval mortality rate showed significant differences between the extract types (**Table 5**). The n-hexane extract showed the highest larvicidal potential when compared with the other two types of extract, with a high mortality rate. Methanol and ethyl acetate extracts have an equivalent effect wtih a low mortality rate difference. Nevertheless, the three types of extracts resulted in a significantly different mortality rate.

In detail, the differences of larvicidal activity of *D. elliptica* root extract were shown by the knockdown larvae in each extract type based on the concentration and exposure time (**Fig.3**). The n-hexane extract showed the highest and fastest larvicidal activity since the initial exposure time and the lowest concentration, even reaching a 100% knockdown rate at the concentration of 10 mg/l. The different condition was shown by the methanol and ethyl-acetate extracts, where the significantly increasing of larvicidal activity was started by the concentration of 25 mg/l, and progressively increase in the higher concentrations. However, an average of 100% larval mortality rate was not reached by the methanol and ethyl-acetate extract types.

Discussion

Toxicity of *D. elliptica* has been used in human life since the last century, from the traditional way as the fish and plant pest poisons[11,12] to the secondary metabolites isolation[13-17,18-20]. This study is part of the exploration in finding the larvicide bioactive compounds from *D. elliptica* which has been carried out in the last century with variations in yield according to solvent extraction, habitat conditions, and geographical regions. The use of the sequential extraction and fractionation method[33-35] which was modified and guided by bioassay test successively produced crude extract methanol, and its derived-extracts from n-hexane (non-polar), ethyl acetate (semi-polar), and water (polar) fractions. Each extract type was subjected to a bioassay test and showed different toxicity against *Ae. aegypti* larvae. This strategy was carried out to obtain the maximum fraction and type of extract from limited raw material[35] so it was more efficient when compared to the parallel method. Extraction in parallel with different solvents shows variations in the percentage of extract weight. Water solvents produce the highest proportion of extracts compared to ethyl acetate, ethanol, and hexane[38,39], while other findings show that methanol

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produces a greater proportion than ethanol and water[40] so that we used the methanol in the initial extraction.

Overall, the results of the bioassay test showed that three extract types of *D. elliptica* are effective compounds because they had high toxicity against *Ae. aegypti* larvae. Previous studies categorized the effectiveness of plant extract larvicides into four levels, namely less effective (LC_{50}>750 mg/l), effective (LC_{50}=100-750 mg/l), moderate (LC_{50}=50-100 mg/l) and high (LC_{50}<50 mg/l) larvicidal activity[22]. In this study, three types of extracts namely n-hexane, methanol, and ethyl acetate have high larvicidal toxicity, so that further research and development become a technical grade of larvicide are still underway. The mortality rate of *Ae. aegypti* larvae in the bioassay test was caused by the exposure of *D. elliptica* root extracts. It is proven that there are no dead larvae in the negative control (aqua dest) and 100% of larvae die in positive control. Based on the WHO standard procedure, if larval mortality in the control group was less than 20%, the results of the bioassay test can still be accepted after being corrected with the Abbott's formula[37].

The n-hexane extract type causes the highest mortality rate of *Ae. aegypti* larvae compared with the other extract types since the beginning of the exposure time and progressively continues to increase for up to 24 hours. The 97.6% mortality rate of *Ae. aegypti* larvae were achieved at a concentration of 10 mg/l. The toxicity of n-hexane extract is related to secondary lipophilic metabolites contained in this extract type[39]. Phytochemical screening shows that there are nine secondary metabolites found in n-hexane extract types. This extract type has never been used in the previous study in the context of the larvicidal activity test of *D. elliptica* against *Ae. aegypti* larvae. Although the toxicity of n-hexane extract is lower than petroleum ether extracts[24], this solvent has resulted in a promising extract.

Larvicidal toxicity test of ethyl acetate extract of *D. elliptica* root against *Ae. aegypti* larvae have not been performed yet. A study explored several classes of chemical compounds from *D. elliptica*, which are bound by ethyl acetate solvents namely alkaloids, flavonoids, sterols, tannins, and triterpenoids[41], and tested for antimicrobial activity. The FTIR analysis of this study indicated the same lead compounds between n-hexane extract and ethyl acetate.

The methanol extract in this study showed high larvicidal toxicity with LC_{50} which was almost equivalent to previous findings in Thailand[22], but lower than findings in India[24]. This solvent is polar and it was used as an initial extraction so that it can bind many classes of chemical compounds to a broad polarity spectrum[35,42]. Phytochemical screening results indicate six classes of chemical compounds contained in the methanol extract.

FTIR results show that the most important differences in the classes of lead compounds in the three types of extracts are stilbenes, isoflavones, and polyhydroxy acids. The toxicity of stilbenoids to mosquito larvae is determined by its lipophilic level[43], whereas lipophilic compounds are bound by hexane solvents. This result indicates that stilbenoids are important chemical compounds in n-hexane extract.

Water extracts have not indicated the larvicidal toxicity against the 3rd instar larvae of *Ae. aegypti* up to the concentration of 1,000 mg/l, whereas the other extract types have killed 100% of larvae at the concentration of 100 mg/l. The possible reasons are the complexity or low levels of chemical compounds bound by this universal solvent. If the bound compounds are very complex and contain many types of chemical compounds, there is a possibility of an antagonistic mechanism between the compounds[44,45]. The second possible reason is that the water extract resulted from the last fraction so that the extract contains only a few remaining compounds, both types, and levels. Although water is the universal extraction solvent, this extract only contains

the polar chemical compounds bound to methanol and can be bound by water, at a low level[46]. Both of these conditions are still unclear and interesting for further study.

In the context of exploration and testing of the larvicidal activity of *D. elliptica* extracts against *Ae. aegypti* larvae in the last two decades, at least six solvents have been used by researchers, namely petroleum ether, methanol, ethanol, and a combination of methanol: chloroform and methyl-chloride: methanol[22-26]. The bioassay test of these extract types showed various results. Petroleum ether extract provides a different effect based on the geographical origin of plant habitat where in Thailand shows LC_{50} of 11.17 mg/l, whereas in India 0.616 mg/l. The larvicide toxicity test of methanol extract was carried out in Thailand and Indonesia (in this study) with equivalent results respectively 13.17 and 14.066 mg/l. This phenomenon is due to differences in levels of secondary metabolites of *D. elliptica* among regions[24,29-32]. The toxicity of plant extract from a combination of methanol-chloroform solvent was more effective than methyl chloride-methanol with LC_{50} of 4.21 and 24-32 mg/l, respectively. This condition showed that the solvent types affected the dissolved secondary metabolites[28].

Exploration of various bioactive larvicidal ingredients from the roots of *D. elliptica* is an important effort in the context of Dengue vector control considering that the temephos resistance of *Ae. aegypti* larvae are increasingly widespread and have been reported in various endemic areas of Dengue[6-8]. Based on these conditions, community attention has increased on the natural insecticides and larvicides compounds because their advantages are easily decomposed and bioaccumulation does not occur in the environment[9]. Bioassay tests on extracts from several types of plants, including *D. Elliptica* have found promising results where the findings present the varied but low LC_{50}[22,24,47]. A limitation that should be noted is that the subject of this study uses the susceptible strains of *Ae. aegypti* larvae and has not included the control group of

temefos-resistant larvae. Further studies are necessary to be conducted to (1) determine the larvicidal potential of these extracts against resistant larvae, (2) isolate and characterize the pure or specific compounds from the extract with the highest toxicity, and (3) formulate the technical grade larvicide guided with bioassay test against the 3rd instar larvae of *Ae. aegypti* both in susceptible and temephos-resistant strains.

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Conclusion

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Three of four fractions of *D. elliptica* extract have high larvicidal toxicity against the 3rd instar larvae of *Ae. aegypti*, namely n-hexane, methanol and ethyl acetate, respectively. The highest toxicity of n-hexane extract is related to the lipophilic compounds contained, and stilbene is thought to play a role in this case. FTIR analysis indicated that n-hexane and ethyl acetate extract contain similar lead compounds while the stilbenes, isoflavones, and polyhydroxy acid were not found in methanol and water extract.

Conflict of Interest

Authors of this paper have no conflict of interest.

Authors' contributions

Sayono Sayono constructed the main idea of the study, supervise all of the research activities, thoroughly analyzed the data, and wrote the main contents of the manuscript. Risyandi Anwar carried out the phytochemical analysis and extraction of *D. elliptica* roots in the natural chemistry laboratory, and supported manuscript draft related to the extraction process and

results. Didik Sumanto carried out the mosquito rearing and larvicidal bioassay and supported the manuscript draft about the bioassay test. All authors discussed the final manuscript.

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Significance statements

This study found the different larvicidal activity in three of four types of tuba root extract that can be beneficial for obtaining the specific chemical compounds as larvicide material for Aedes mosquitoes. This study will help the researchers to uncover critical areas of finding alternative methods for solving the resistance problems in mosquito vector control that many researchers are unable to explore. This finding reinforces that new theories on herbal chemical compounds can be arrived at the near times.

References

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1. Kraemer, M.U., M.E. Sinka, K.A. Duda, A.Q.N. Mylne, and F.M. Shearer, et al, 2015. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. Albopictus*. *Elife*, 4: e08347. doi: 10.7554/eLife.08347.

2. WHO, 2019. Dengue and Severe Dengue. Geneva, Switzerland. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue. Accessed on September 15, 2019.
3. Pérez, D., P. Lefèvre, L. Sánchez, L.M. Sánchez, M. Boelaert, G. Kourí, P.V. der Stuyft, 2007. Community participation in *Aedes aegypti* control: a sociological perspective on five years of research in the health area ‘‘26 de Julio’’, Havana, Cuba. Trop. Med. Int. Health., 12(5):664–672. doi:10.1111/j.1365-3156.2007.01833.x.
4. Sayono, S., W. Widoyono, D. Sumanto, R. Rokhani, 2019. Impact of Dengue Surveillance Workers on Community Participation and Satisfaction of Dengue Virus Control Measures in Semarang Municipality, Indonesia: A Policy Breakthrough in Public Health Action. Osong Public Health Res Perspect. 10(6):376-385. doi: 10.24171/j.phrp.2019.10.6.08
5. George, L., A. Lenhart, J. Toledo, A. Lazaro, and W.W. Han, et al, Community-Effectiveness of Temephos for Dengue Vector Control: A Systematic Literature Review. PLoS Negl. Trop. Dis., 9(9): e0004006, 2015. doi:10.1371/journal.pn-td.0004006
6. Moyes, C., J. Vontas, A.J. Martins, L.C. Ng, S.Y. Koou, and I. Dufour, et al., 2017. Contemporary status of insecticides resistance in the major Aedes vectors of arbociruses infecting humans. PLoS Negl. Trop. Dis., 11(7):e0005625. Doi: 10.1371/journal.pntd.0005625
7. Mulyatno, K.C., A. Yamanaka, Ngadino, E. Konishi, 2012. Resistance of *Aedes aegypti* (Lin.) larvae to temephos in Surabaya, Indonesia. Southeast Asian J. Trop. Med. Public Health, 43(1):29–33. PMID: 23082551
8. Ikawati, B., Sunaryo, Wahyudi, B. Fajar, 2017. *Aedes aegypti* Resistance to Temephos in Central Java, Indonesia. Adv. Sci. Lett., 23(4):3544–3546. DOI**:** https://doi.org/10.1166/asl.2017.9163

9. Arnason, J.T., S.R. Sims, I.M. Scott, 2012. Natural products from plants as insecticides. In: Phytoc-hemistry and Pharmacognosy. Encyclopedia of Life Support System (EoLSS). Available from: https://www.eolss.net/Sample-Chapters/C06/E6-151-13.pdf
10. Starr, F., K. Starr, L. Loope, 2003. *Derris elliptica*: *poison vine, fabaceae*; 1-4. Available from: http://www.hear.org/starr/hiplants/reports/pdf/derris_elliptica.pdf
11. Orwa, C., A. Mutua, R. Kindt, R. Jamnadass, S. Anthony, 2009. Agroforestree Database: a tree reference and selection guide version 4.0. available from: http://www.worldagroforestry.org/sit-es/treedbs/treedatabases.asp)
12. Sirichamorn, Y., F.A.C.B. Adema, B. Gravendeel, P.C. Welzen, 2012. Phylogeny of palaeotropic *Derris*-like taxa (Fabaceae) based on chloroplast and nuclear DNA sequences shows reorganization of (infra) generic classifications is needed. Am. J. Bot., 99(11):1793–1808. http://doi.org/10.3732/ajb.1200390.
13. Morel, S., J.J. Helesbeux, D. Séraphin, S. Derbré, J. Gato, and M-C. Aumond, et al., 2013. Anti-AGEs and antiparasitic activity of an original prenylated isoflavonoid and flavonone isolated from *Derris ferruginea*. Phytochem. Lett., 6:498–503. http://dx.doi.org/10.1016/j.phytol.2-013.06.002.
14. Wu, X., Z. Song, H. Xu, H. Zhang, and W. Chen, et al., 2012. Chemical constituents of the aerial part of *Derris elliptica*. Fitoterapia, vol. 83, no. 4, pp. 723–736, 2012. doi:10.1016/j.fitote.2012.02.2015.
15. Musa, W., J. Ahmad, C.J. Lamangantjo, 2016. Bioactive Compounds in Tombili Seeds and Tubile Roots as the Alternative for Synthetic Pesticide to Protect Wheats from Insects and Pests. Int. J. ChemTech Res., 9(4):604–615. http://www.sphinxsai.com/2016/ch_vol9_n-o4/3/(604-616)V9N4CT.pdf

16. Lu, H.Y, 2008. Isoflavones and their derivatives from the root of *Derris elliptica* (Roxb.) Benth. Chin. J. Nat. Medicines. 6(4):265–267. DOI:10.3724/ SP.J.1009.2008.00265
17. Russel, D.A., W.J.S. Fong, G.G. Twigg, H.F. Sore, and D.R. Spring, 2017. Stereocontrolled semisyntheses of elliptone and 12aβ-Hydroxyelliptone. J. Nat. Prod., 80(10):2751-2755. https://doi.org-/10.1021/acs.jnatprod.7b00527
18. Seiferle, E.J., D.E.H. Frear, 1948. Insecticides derived from plants. Ind. Eng. Chem., 40(4):683–691. https://doi.org/10.1021/ie50460a022
19. Gupta, R.C., 2007. Rotenone, in Veterinary Toxicology: Basic and Clinical Principles, Academic Press. https://doi.org/10.1016/B978-012370467-2/50139-5
20. Yoon, A.S., 2009. Extraction and Formulation Development of Derris elliptica for Insect Pest Control. [Ph.D Thesis]. Prince of Songkla University, Thailand. http://kb.psu.ac.th/psukb/bitstream/2010/7072/1/316615.pdf
21. Zubairi, S.I., M.R. Sarmidi, R.A. Aziz, 2014. Bio-Active Constituents of Rotenoids Resin Extracted from Derris elliptica Roots: Comparison between Local Plant Extract and SAPHYR (France) Cube Resin. Adv. Environ. Biol., 8(4):904–909 www.aensiweb.com/old/aeb/2014/904-909.pdf
22. Komalamisra, N., Y. Trongtokit, R. Rongsriyam, and C. Apiwathnasorn, 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. https://www.tm.mahidol.ac.th/seameo/2005_36_6/09-3546.pdf
23. Zubairi, S.I., M.R. Sarmidi, and R.A. Aziz, 2015. A preliminary study on mosquito larvicidal efficacy of rotenone extracted from Malaysia Derris sp. J. Teknol., 76(1):275–279. Available from www.jurnalteknologi.utm.my

24. Dohutia, C., D.R. Bhattacharyya, S.K. Sharma, P.K. Mohapatra, and K. Gogoi, et al., 2015. Larvicidal activity of few select indigenous plants of North East India against disease vector mosquitoes (Diptera: Culicidae. Trop. Biomed., 32(1):17–23. Available: https://www.ncbi.nlm.nih.gov/pubmed/25801251

25. Sayono, U. Nurullita, M. Suryani, 2010. Pengaruh konsentrasi flavonoid dalam ekstrak akar tuba (*Derris elliptica*) terhadap kematian larva Aedes aegypti. J. Kesehat. Masy. Indones., 6(1):38-47. https://www.e-jurnal.com/2014/11/pengaruh-konsentrasi-flavonoid-dalam.html

26. Komansilan, A., N.W. Suriani, H.T. Lawalata, 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

27. Sayono, S., A. Permatasari, D. Sumanto, 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.

28. Nguyen, A., Y. Phan, 2014. Preliminary phytochemical analysis of different solvent extracts of Derris elliptica (Roxb.) Benth leaves. International Journal of innovation and Applied Research, 2(12):74–76. Available from http://www.journalijiar.com.

29. Karahan, F., C. Avsar, I.I. Ozyigit, et al., 2016. Antimicrobial and antioxidant activities of med-icinal plant *Glycyrrhiza glabra* var: *gladulifera* from different habitats. Biotechnol. Biotech. Eq., 30(4):797–804. https://doi.org/10.1080/ 13102818.2016.1179590

30. Kumar, S., A. Yadav, M. Yadav, and J.P. Yadav, 2017. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm.f. BMC Res. Notes, 10(60). DOI 10.1186/s13104-017-2385-3

31. Sampio, B.L., F.B. Da-Costa, 2018. Influence of abiotic environmental factors on the main cons-tituents of the volatile oils of *Tithonia diversifolia*. Rev. Bras. Farmacogn., 28(2):135–144. https://doi.org/10.1016/j.bjp.2018.02.005
32. Qi, M., X. Hua, X. Peng, X. Yan, and J. Lin, 2018. Comparison of chemical composition in the buds of *Aralia elata* from different geographical origins of China. R. Soc. Open Sci., 5:180676. http://dx.doi.org/10.1098/rsos/180676.
33. Li, J.X., T. Hareyama, Y. Tezuka. Y. Zhang, T. Miyagara, and S. Kadota, 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
34. Koehn, F.E., G T. Carter, 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov., 4(3):206–220. doi:10.1038/nrd1657
35. Liu, Z., 2008. Preparation of botanical samples for biomedical research. Endocr. Metab. Immune Disord. Drug Targets, 8(2):112–121. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936020/pdf/nihms556279.pdf
36. Walter Reed Biosystematics Unit. Mosquito identification keys. http://www.wrbu.org/aors/pacom_Keys.html
37. WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides, Geneva, Switzerland:7-12. Available from https://apps.who.int/iris/h-andle/10665/69101
38. Razak, M.F.B.A., P.K. Yong, Z.M. Shah, L.C. Abdullah, S.S. Yee, and I.T.C.S. Yaw, 2012. The effect of varying solvent polarity on extraction yield of *Orthosiphon stamineus* leaves. J. Appl. Sci., 12(11):1207–1210.
39. Abarca-Vargas, R., C.F.P. Malacara, V.L. Petricevich, 2016. Characterization of Chemical Compounds with Antioxidant and Cytotoxic Activities in Bougainvillea x buttiana Hol-ttum

and Standl, (var. Rose) Extracts. Antioxidants, 5(45):1-11. doi:10.3390/anti-ox5040045. www.mdpi.com/journal/antioxidants.

40. Borah, R., S.P. Biwas, 2018. Tulsi (Ocimum sanctum), excellent source of phytochemicals. Int. J. Environ. Agric. Biotech., 3(5):1732-1738. http://dx.doi.org/10.22161/ijeab/-3.5.21
41. Khan, M.R., A.D. Omoloso, Y. Barewai, 2006. Antimicrobial activity of the Derris elliptica, Derris indica and Derris trifoliata extractives. Fitoterapia, 77(4):327-330. doi:10.1016/j.-fitote.2006.03.007
42. Tiwari, P., B. Kumar, M. Kaur, and H. Kaur, 2011. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia, vol. 1, no. 1, pp. 98-106, 2011. Available online from http://www.ipharmsciencia.com
43. Sobolev, V.S., S.I. Khan, N. Tabanca et al., 2011. Biological activity of peanut (Arachis hypogaea) phytoalexins and selected natural and synthetic stilbenoids. J. Agric. Food Chem., 59(5):1673-1682. doi:10.1021/jf104742n
44. Milugo, T.K., L.K. Omosa, O.J. Ochanda, B.O. Owuor, and F.A. Wamunyokoli, et al., 2013. Antagonistic effect of alkaloids andsaponins on bioactivity in the quinine tree (Rauv-olfia caffrasond.): further evidence to support biotechnology in traditional medicinal plants. BMC Complement. Altern. Med., 13(285):285. doi:10.1186/1472-6882-13-285
45. Celep, E., Y. İnan, S. Akyüz, and E. Yesilada, 2017. The bioaccessible phenolic profile and antioxidant potential of Hypericum perfoliatum L. after simulated human digestion. Ind. Crops Prod., 109:717-723. http://dx.doi.org/10.1016/j.indcrop.2017.09.032
46. Dawan, D., J. Gupta, 2017. Comparison of Different Solvents for Phytochemical Extraction Potential from Datura metel Plant Leaves. Int. J. Biol. Chem., 11(1)::17-22. doi:10.3923/i-jbc.2017.17.22

47. Scalvenzi, L., M. Radice, L. Toma, F. Severini, and D. Boccolini, et al., 2019. Larvicidal activity of *Ocimum campechianum*, *Ocotea quixos* and *Piper aduncum* essential oils against *Aedes aegypti*. Parasite, 26(23):1-8. https://doi.org/10.1051/parasite/2019024.

Table 1. Mortality rate of mosquito larvae after 24 h exposed of *D. elliptica* extracts in the initial bioassay

Dosage (mg/L)	Extract types			
	Water	Methanol	Ethyl acetate	n-hexane
50	0	98.4	86	98
100	0	99.2	98	100
500	0	100	100	100
1,000	0	100	100	100

Table 2. FTIR analysis of four extract types of Derris elliptica root indicates nine phytochemical compounds.

Lead compounds	Extract types			
	Methanol	n-Hexane	Ethyl acetate	Distilled-water
Rotenone	+	+	+	+
Pterocarpans	+	+	+	-
Cumestans	+	+	+	-
Flavone	+	+	+	-
Anthraquinone	+	+	+	-
Ceramides	+	+	+	+
Stilbenes	-	+	+	-
Isoflavones	-	+	+	+
Poly-hydroxy acids	-	+	+	+

+ = Present and - = Absent

Table 3. Mortality rate of the 3rd instar larvae of *Aedes aegypti* based on the extract types and concentrations of the *Derris elliptica* root extract

Extract types	**Concentration (mg/l)**	**Minimum**	**Maximum**	**Mean**	**Std. deviation**
Methanol	3	0	4	1.6	2.19
	5	4	24	12.8	9.12
	10	12	32	21.6	7.79
	25	68	92	80.0	8.94
	50	96	100	98.4	2.19
	100	96	100	99.2	1.79
Ethyl acetate	3	0	4	1.6	2.19
	5	4	8	4.8	1.79
	10	12	24	16.8	4.38
	25	48	64	54.4	6.07
	50	80	92	86.4	4.56

	100	96	100	98.4	2.19
n-Hexane	3	4	32	18.4	12.84
	5	52	88	75.2	13.98
	10	96	100	97.6	2.19
	25	100	100	100	0.00
	50	100	100	100	0.00
	100	100	100	100	0.00
Negative control (-)	-	0	0	0	0
Positive control (+)	0.02	100	100	100	0

1 (-) aquadest; (+) temephos 0.02 mg/l
2

Table 4. Results of Probit analysis showed the LC_{50} and LC_{90} of the extract toxicity of *Derris elliptica* root against the 3rd instar larvae of *Aedes aegypti*

Extract types	Regression equation	Lethal Concentration (mg/l)		Chi Square	p
		LC50 (95% confidence limits)	LC90 (95% confidence limits)		
Methanol	Y= -3.689+3.213X	14.066 (10.700 – 18.755)	35.237 (25.217 – 61.023)	15.004	0.005
Ethyl acetate	Y= -3.725+2.815X	21.063 (18.987 – 23.389)	60.096 (51.814 – 71.695)	2.764	0.598
n-Hexane	Y= -3.637+5.950X	4.086 (3.825 – 4.355)	6.709 (6.144 – 7.603)	4.530	0.339

Table 5. Pairwise comparisons of larval mortality based on the extract types

Pairwised extract types	Mean difference	p	95% confidence interval for difference
Methanol >< Ethyl acetate	8.533	0.035	0.611 – 16.455
n-Hexane >< Ethyl acetate	38.133	0.000	30.211 – 46.055
n-Hexane >< Methanol	29.600	0.000	21.678 – 37.522

Figures

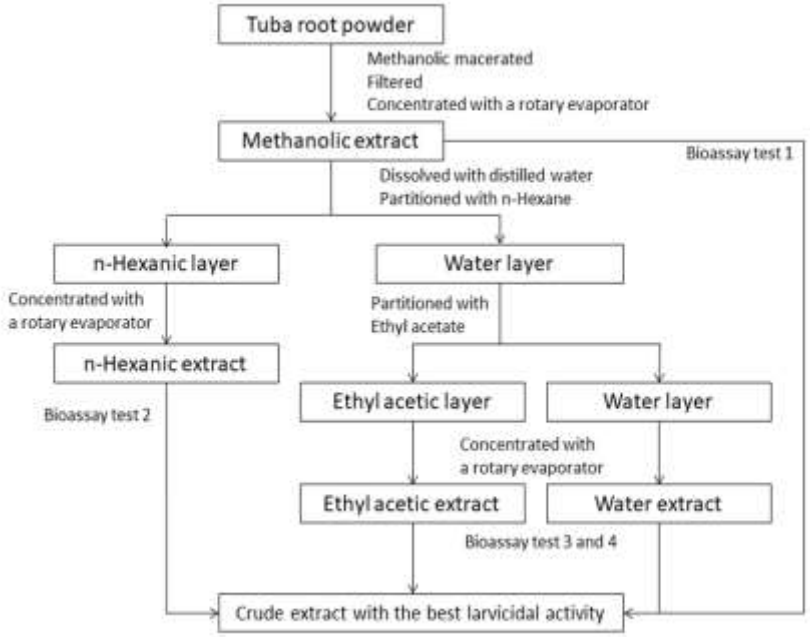

Fig.1. Steps of the extraction and sequential fractionation of *Derris elliptica* root using four different polarity solvents.

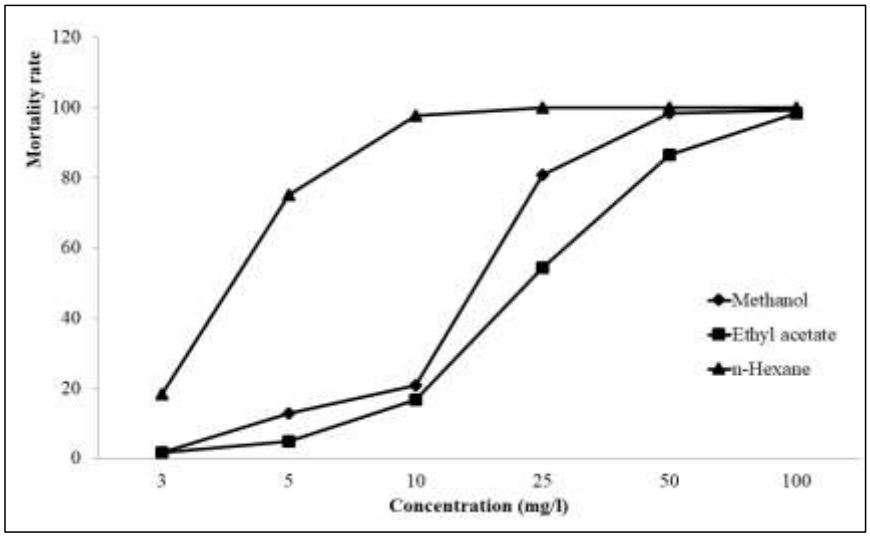

Fig.2. Trend of mortality rate of *Ae. aegypti* larvae based on concentration and types of Tuba root extract. The n-hexane extract type resulted the highest mortality rate of mosquito larvae.

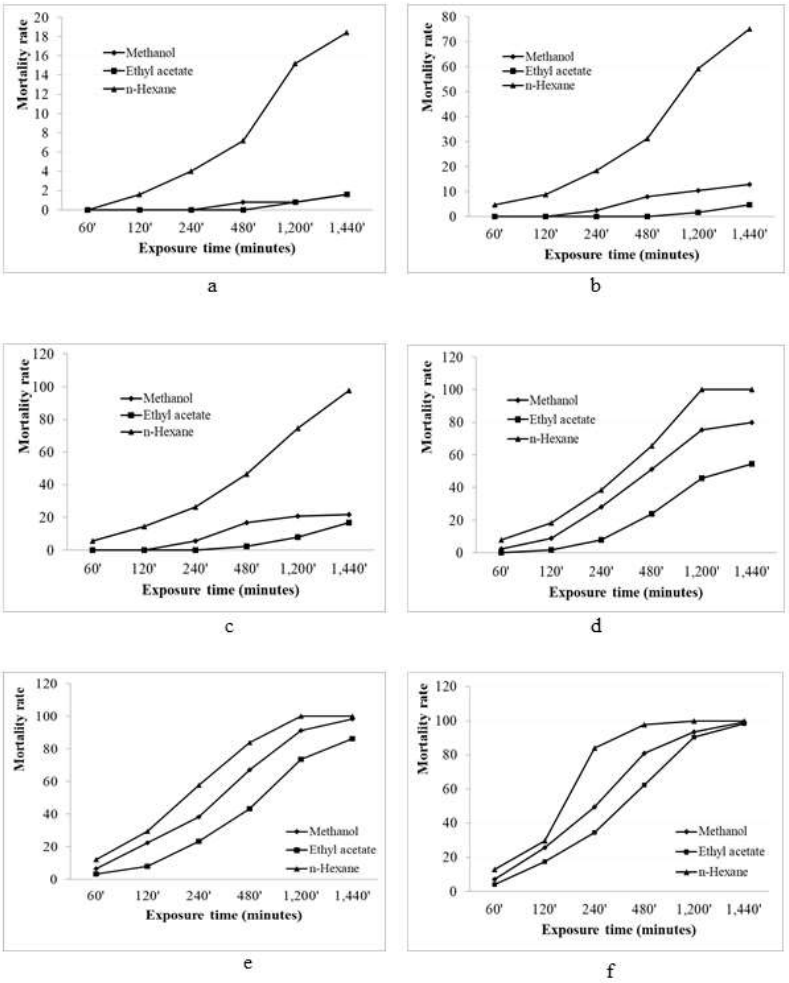

Fig.3. The knockdown rate of *Ae. aegypti* larvae based on the different extract types, exposure times and concentrations (a =3 mg/l, b =5 mg/l, c =10 mg/l, d =25 mg/l, e =50 mg/l, and f =100 mg/l).

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Research Article
Evaluation of Toxicity in Four Extract Types of Tuba Root against Dengue Vector, *Aedes aegypti* (Diptera: Culicidae) Larvae

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Abstract

Background and Objective: Since the Dengue virus spreads rapidly and the vector becomes resistant to insecticides and larvicides, exploration of new compounds that overcome resistance problems, are easily degraded and do not lead to bioaccumulation, is needed. This study evaluated four extract types of *Derris elliptica* represented the polar, semi-polar and nonpolar extract against the 3rd-instar larvae of *Ae. aegypti* and determined the effective concentration among the extracts. **Materials and Methods:** The crude extract was obtained from the maceration of root powder of the plant with methanol and subsequently evaporated. The crude extract was diluted in distilled water and partitioned sequentially with ethyl-acetate, n-hexane and water to obtain their fractions. All the fractions were evaporated to obtain their extract types. Initial bioassay test of the extracts with concentration ranges of 50, 100, 500 and 1,000 mg L^{-1} against *A. aegypti* larvae and resulted in 86-100% larval mortality rates at concentrations of 50 and 100 mg L^{-1}, except for water extract. The lower concentration range of 3, 5, 10, 25, 50 and 100 mg L^{-1} of three extract types were tested. **Results:** Larval mortality rates of 18.4-100, 1.6-99.2 and 0.8-98.4% with LC_{50} of 4.088, 14.066 and 21.063 mg L^{-1}, respectively for n-hexane, methanol and ethyl-acetate. FTIR analysis indicated nine lead compounds in which rotenone and ceramides were observed in all extract types. **Conclusion:** The n-hexane extract showed the highest larvicidal toxicity and its specific compounds are necessarily isolated to obtain pure bioactive ingredients.

Key words: Larvicidal potency, *Derris elliptica*, *Aedes aegypti*, tuba root extract, n-hexane

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Since the Dengue virus spread rapidly in the past five decades from nine countries in 1970 to 128 countries in the tropics and subtropics recently[1,2], community attention and involvement in Dengue endemic areas in controlling *Aedes aegypti* mosquitoes have increased[3,4]. Aedes mosquito larvae become a strategic target in the Dengue vector control, where temephos rely upon larvicides. The campaign to use temephos is done seriously following and combining with the other methods to control the adult and larval stage of Aedes mosquitoes. This phenomenon occurs widely and intensively in Dengue endemic areas throughout the world for a long time[5] and results in the emergence of resistant strains of *Ae. aegypti* larvae against temephos, which have been reported in many countries[6], including in Indonesia[7,8].

The development of *Ae. aegypti* larvae resistance to temephos has hampered the Dengue vector control program. This condition triggers the researcher to find the new chemical compounds that are effective, biodegradable and do not cause bioaccumulation in environment[9]. In line with these efforts, the utilization of the potential for tubal root toxicity has evolved from traditional to modern methods in solving the problem of controlling dengue.

Tuba (*D. elliptica* (Wallich) Benth) is a poisonous vine that is easily found on uncultivated agricultural land. This plant grows in the South Asian, Southeast Asian and Hawaiian regions[10]. Traditionally, the tuba roots have long been used by residents of the regions as a fish poison and plant pest pesticide[11,12]. The use of tuba root is related to chemical compounds contained in the plant comprising isoflavonoids[13], flavonoids[14,15], ceramides and polyhydroxy acids[16], as well as rotenoids[17] which include compounds such as rotenone, deguelin, toxicarol, sumatrol, elliptone and malaccol[18-21].

Previously, the studies on the larvicidal toxicity of tuba root extract against *Ae. aegypti* larvae rapidly develop in several regions to find the new active compound of larvicide. A study in Thailand found that tuba root extract with petroleum ether (PE) and methanol solvents showed different toxicity, where the PE extract showed a lower lethal concentration of 50% of mortality LC_{50} and LC_{90} rather than the others, namely[22] 11.17 and 27.74 mg L^{-1}. Two other studies in two different countries tested the *D. elliptica* root extract that was resulted from a combination of two solvents. In Malaysia, a 1:1 combination of methyl-chloride and methanol results in higher toxicity rather than 1:9 combination against mosquito larvae with LC_{50} of 24 and 32 mg L^{-1}, respectively[23]. In India, a study found that PE extract of tuba root also resulted in higher toxicity against *Aa. aegypti* larvae than the combination of the methanol-chloroform extract with LC_{50} of 0.616 and 4.21 mg L^{-1}, respectively[24]. Similar studies have also been reported from Indonesia. A study on the toxicity of liquid ethanolic extract of tuba root against the filial one (F1) larvae of wild-caught *Ae. aegypti* larvae showed that the concentration 0.5% caused 86% of mortality rate[25], while another study using the ethanolic extract of tuba root showed the higher larvicidal potency against the laboratory strain of *Ae. aegypti* larvae with[26] LC_{50} of 47.7526 mg L^{-1}. But the temephos-resistant *Ae. aegypti* larvae needed a higher effective concentration of methanolic extract of tuba root with the LC_{50} and LC_{90} were 1,600 and 2,040 ppm, respectively[27]. These studies indicated that *D. elliptica* root extract has a variation of toxicity based on the extraction of solvents and habitat geographic origin. Another study showed that the number and type of secondary metabolites were influenced by the extraction solvent[28], while the composition of chemical constituents is affected by environmental habitat and climate conditions[29-32]. Based on the phenomenon, this *in-vitro* study aims to obtain the highest toxicity and the effective concentration of the local *D. elliptica* extract against the 3rd-instar larvae of laboratory strain *Ae. aegypti* based on the distilled water, methanol, ethyl acetate and n-hexane extract types.

MATERIALS AND METHODS

Study area: This study was conducted in eight months from March to October 2019. Tuba roots were taken from uncultivated lands in the hilly areas of the Samping village of Kemiri sub-district of Purworejo district, Central Java Province, Indonesia. The extraction process was conducted in the Natural Chemical Laboratory of Sciences and Mathematics Faculty of Garut University, West Java Province while the mosquito rearing and bioassay tests were conducted in the Epidemiology and Tropical Diseases Laboratory of Public Health Faculty of Universitas Muhammadiyah Semarang, Indonesia.

Plant collection and extraction: The vine stems of Tuba plants in the ground were gently pulled out so that the roots did not break. The base of roots was cut, cleaned and dried in the shade, before being sent to the laboratory for the extraction process. The study used the previous procedure of extraction and fractionation[33-35] with modification (Fig. 1). Briefly, the crude extract was obtained by maceration of six kilograms of tuba root dry powder in methanol for 3×24 h. The filtrate was separated from the residue and evaporated to produces 400 g of methanol extract. The polarity of the extract was separated by the liquid-liquid partition method. As much as 250 mL of aquadest was added to 120 g of solid

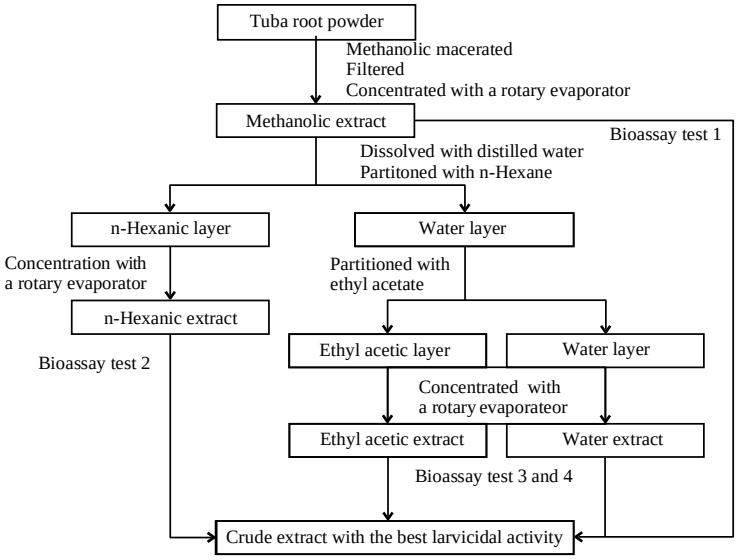

Fig. 1: Steps of the extraction and sequential fractionation of *D. elliptica* root using four different polarity solvents

methanol extract and stirred until completely homogeneous. Homogenate was entered into a 500 mL separation funnel and 250 mL of n-hexane was added to separate the low polarity compounds, then shaken until it completely separates the top layer (n-hexane phase) and the bottom (water phase). The top and bottom layers were separated. The top layer was evaporated to obtain the solid n-hexane extract. In the lower part, ethyl acetate was added to separate the semi-polar compounds. The mixture was processed with a separation funnel like the previous procedure to obtain the upper layer (ethyl-acetate phase) and the lower (water layer). Both fractions were evaporated separately to obtain the solid ethyl acetic and water extracts. A part of the four types of extracts was prepared for larvicidal activity (bioassay) test and phytochemical analysis using the Fourier Transform Infrared (FTIR) spectrophotometer.

Experimental mosquitoes: The parental *Ae. aegypti* mosquitoes were obtained in the larval stage from Sendang Mulyo village of Blora district, Central Java Province, Indonesia. Larvae were maintained to be the adult mosquito in the Epidemiology and Tropical Diseases Laboratory. To obtain thousands of larvae with the same age, the parental *Ae. aegypti* mosquito was reared up to the third generation. During the rearing process, mosquitoes were fed with guinea pig's blood and larvae were fed with dog food. The experiment temperature condition was maintained at the range of 25-28EC and humidity of 70-80%. The late third or early of the fourth instar of filial (F3) larvae was subjected to the bioassay test[36].

Larvicidal bioassay: To determine the larvicidal toxicity of the *D. elliptica* root extracts, the WHO guideline was used[34]. Briefly based on the modification of previous study[24], the initial bioassay used four concentration ranges, namely 50, 100, 500 and 1000 mg LG^{1} in 100 mL distilled water for each *D. elliptica* root extract from the four solvents (methanol, n-hexane, ethyl acetate and distilled water) and placed in the plastic cup. Each concentration level was prepared five times replication so that there were a total of 20 cups in each group of extract type. A total of 25 third instar larvae of *Ae. aegypti* were contacted for 24 h with the *D. elliptica* root extract solution in each cup. Two control groups were provided in this experiment, namely 0.02 mg LG^{1} temephos solution as the positive control and distilled water as the negative control. Knockdown larvae of each container were observed in 30, 60, 120, 240, 480 and 1,440 min experiments. Larval mortality in each container was calculated after 24 h of observation. The temperature was maintained at 25-28EC. The initial bioassay showed that the concentration up to 100 ppm causes a range of 96-100% of the mortality rate of *Ae. aegypti* larvae among methanol, n-hexane and ethyl acetate extract types. There were not dead mosquito larvae found in the distilled water extract so that the next step of the bioassay test for this extract type was stopped. Based on the results, it set a new concentration range of 3, 5, 10, 25, 50 and 100 mg LG^{1} for the three extract types, namely methanol, n-hexane and ethyl-acetate.

Statistical analysis: The data of this study are presented descriptively in minimum-maximum, Mean±standard deviation (SD), mean and 95% confidence interval (CI) and analytically in compare means by using two-way analysis of variance (ANOVA). LC_{50} and LC_{90} were determined by using the probit analysis. All the data analysis was performed by the SPSS statistical software.

Ethical approval: Ethics approval of this study was obtained from the Ethics Committee of Health Research of Public Health Faculty of Universitas Muhammadiyah Semarang with registration number 231/KEPK-FKM/UNIMUS/2019.

RESULTS

Extract types and phytochemical compounds: Four extract types, namely methanol, n-hexane, ethyl acetate and distilled water representing the polar, non-polar, semi-polar and high polar extract (Fig. 1) were obtained. Overall, the results of the FTIR analysis indicated nine phytochemical compounds that were distributed to four types of extracts. The findings show that each type of extract can contain several phytochemical compounds that are also found in other types of extracts, although the only rotenone and ceramides were found in all extract types (Table 1). The results indicated that the semi and non-polar solvents can bind more groups of phytochemical compounds.

Initial bioassay test: Based on the experimented concentration ranges of tuba root extract, there were three extract types showed the high toxicity against the 3rd instar larvae of *Ae. aegypti*, except the water extract. Twenty-four- hours exposure of the water extract has not been caused the larval mortality among all of the concentration ranges so that this extract type was excluded from the next experiment. Exposure of the lowest concentration (50 mg L^{-1}) of the n-hexane, methanol and ethyl acetic extract has resulted in the larval mortality rate 98, 98.4 and 86% respectively (Table 2). One hundred percent of the larval mortality rate was reached by the concentration of 100 mg L^{-1} among the three extract types.

Bioassay test with the specific concentration ranges: The six concentration ranges of *D. elliptica* root extract tested show the larval mortality rate of the 3rd-instar larvae of *Ae aegypti* have been found since the lowest concentration (3 mg L^{-1}) and increase directly proportional to the concentration. The range of larval mortality in each extract type was 18.4-100, 1.6-99.2 and 0.8-98.4% for n-hexane, methanol and ethyl- acetate, respectively. Average larval mortality of 100% was only found in the n-hexane extract, even since the concentration was 25 ppm (Table 3). This finding indicated that the n-hexane extract has a higher and faster larvicidal activity rather than the others.

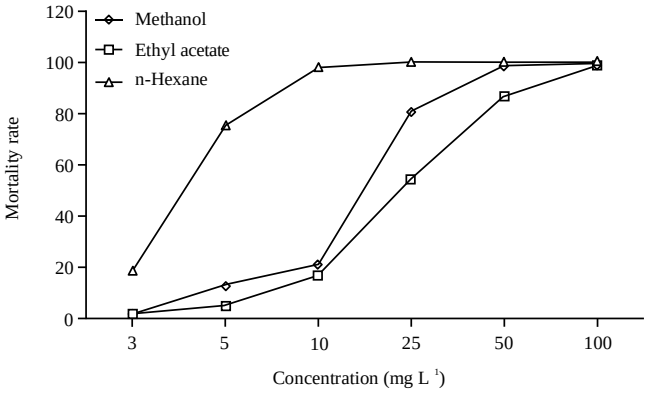

Fig. 2: Trend of mortality rate of *Ae. aegypti* larvae based on concentration and types of Tuba root extract

Table 1: FTIR analysis of four extract types of *D. elliptica* root indicates nine phytochemical compounds

	Extract types			
Lead compounds	Methanol	n-Hexane	Ethyl acetate	Distilled-water
Rotenone	+	+	+	+
Pterocarpans	+	+	+	-
Coumestans	+	+	+	-
Flavone	+	+	+	-
Anthraquinone	+	+	+	-
Ceramides	+	+	+	+
Stilbenes	-	+	+	-
Isoflavones	-	+	+	+
Poly-hydroxy acids	-	+	+	+

+: Present and -: Absent

Table 2: Mortality rate of mosquito larvae after 24 h exposed of *D. elliptica* extracts in the initial bioassay

	Extract types			
Dosage (mg L^{-1})	Water	Methanol	Ethyl acetate	n-hexane
50	0	98.4	86	98
100	0	99.2	98	100
500	0	100.0	100	100
1,000	0	100.0	100	100

Larvicidal activity of the three types of *D. elliptica* root extract also indicated a high effectiveness level, which was shown by LC_{50} and LC_{90} of the probit analysis results of 4,088 and 6,709 mg L^{-1}, 14,066 and 35,237 mg L^{-1} and 21,063 and 60,096 mg L^{-1} for n-hexane, methanol and ethyl-acetate, respectively (Table 4). Overall, the result of the two-way ANOVA test showed the differences of larvicidal activity of the *D. elliptica* root against the 3rd instar larvae of *Ae. aegypti* based on the interaction of the extract types and concentration levels (Fig. 2). Pairwise comparisons of the

Table 3: Mortality rate of the 3rd instar larvae of *Ae. aegypti* based on the extract types and concentrations of the *D. elliptica* root extract

Extract types	Concentration (mg L^{-1})	Minimum	Maximum	Mean	Standard deviation
Methanol	3.0	0	4	1.6	2.19
	5.0	4	24	12.8	9.12
	10.0	12	32	21.6	7.79
	25.0	68	92	80.0	8.94
	50.0	96	100	98.4	2.19
	100.0	96	100	99.2	1.79
Ethyl acetate	3.0	0	4	1.6	2.19
	5.0	4	8	4.8	1.79
	10.0	12	24	16.8	4.38
	25.0	48	64	54.4	6.07
	50.0	80	92	86.4	4.56
	100.0	96	100	98.4	2.19
n-Hexane	3.0	4	32	18.4	12.84
	5.0	52	88	75.2	13.98
	10.0	96	100	97.6	2.19
	25.0	100	100	100.0	0.00
	50.0	100	100	100.0	0.00
	100.0	100	100	100.0	0.00
Negative control (-)	-	0	0	0.0	0.00
Positive control (+)	0.02	100	100	100.0	0.00

-: Aquadest, +: Temephos 0.02 mg L^{-1}

Table 4: Results of Probit analysis showed the LC_{50} and LC_{90} of the extract toxicity of *D. elliptica* root against the 3rd instar larvae of *Ae. aegypti*

		Lethal concentration (mg L^{-1})			
Extract types	Regression equation	LC_{50} (95% confidence limits)	LC90 (95% confidence limits)	Chi square	p-value
Methanol	Y = -3.689+3.213X	14.066 (10.700-18.755)	35.237 (25.217-61.023)	15.004	0.005
Ethyl acetate	Y = -3.725+2.815X	21.063 (18.987-23.389)	60.096 (51.814-71.695)	2.764	0.598
n-Hexane	Y = -3.637+5.950X	4.086 (3.825-4.355)	6.709 (6.144-7.603)	4.530	0.339

Table 5. Pairwise comparisons of larval mortality based on the extract types

Pairwised extract types	Mean difference	p-value	95% confidence interval for difference
Methanol >< Ethyl acetate	8.533	0.035	0.611-16.455
n-Hexane >< Ethyl acetate	38.133	0.000	30.211-46.055
n-Hexane >< Methanol	29.600	0.000	21.678-37.522

larval mortality rate showed significant differences between the extract types (Table 5). The n-hexane extract showed the highest larvicidal potential when compared with the other two types of extract, with a high mortality rate. Methanol and ethyl acetate extracts have an equivalent effect with a low mortality rate difference. Nevertheless, the three types of extracts resulted in a significantly different mortality rate.

In detail, the differences of larvicidal activity of *D. elliptica* root extract were shown by the knockdown larvae in each extract type based on the concentration and exposure time (Fig. 3). The n-hexane extract showed the highest and fastest larvicidal activity since the initial exposure time and the lowest concentration, even reaching a 100% knockdown rate at the concentration of 10 mg L^{-1}. The different condition was shown by the methanol and ethyl-acetate extracts, where the significantly increasing of larvicidal activity was started by the concentration of 25 mg L^{-1} and progressively increase in the higher concentrations. However, an average of 100% larval mortality rate was not reached by the methanol and ethyl-acetate extract types.

DISCUSSION

Toxicity of *D. elliptica* has been used in human life since the last century, from the traditional way as the fish and plant pest poisons[11,12] to the secondary metabolites isolation[13-17,18-20]. This study is part of the exploration in finding the larvicide bioactive compounds from *D. elliptica* which has been carried out in the last century with variations in yield according to solvent extraction, habitat conditions and geographical regions. The use of the sequential extraction and fractionation

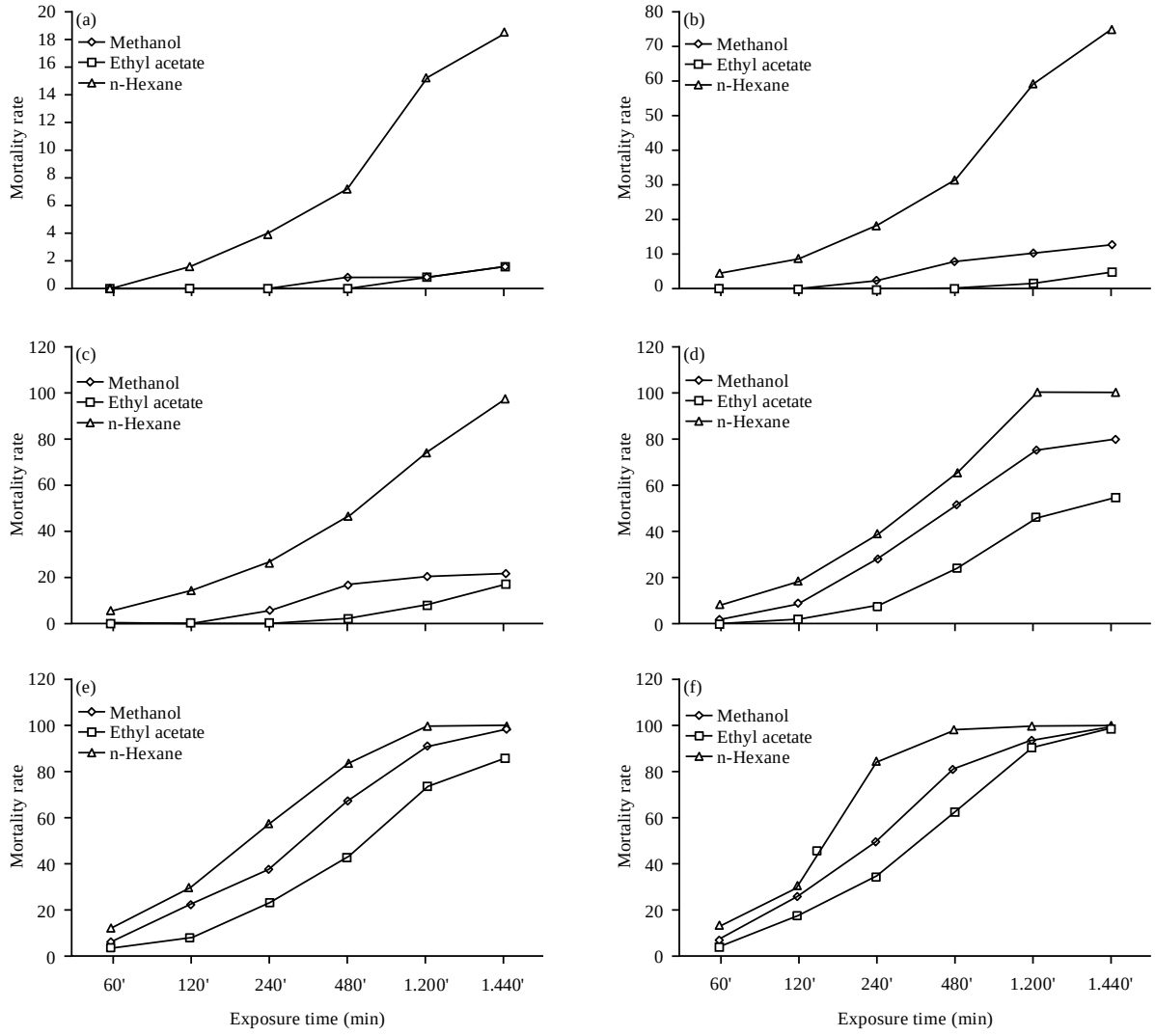

Fig. 3(a-f): The knockdown rate of *Ae.aegypti* larvae based on the different extract types, exposure times and concentrations (a) 3 mg LG^1, (b) 5 mg LG^1, (c) 10 mg LG^1, (d) 25 mg LG^1, (e) 50 mg LG^1 and (f) 100 mg LG^1)

method[33-35] which was modified and guided by bioassay test successively produced crude extract methanol and its derived-extracts from n-hexane (non-polar), ethyl acetate (semi-polar) and water (polar) fractions. Each extract type was subjected to a bioassay test and showed different toxicity against *Ae. aegypti* larvae. This strategy was carried out to obtain the maximum fraction and type of extract from limited raw material[35] so it was more efficient when compared to the parallel method. Extraction in parallel with different solvents shows variations in the percentage of extract weight. Water solvents produce the highest proportion of extracts compared to ethyl acetate, ethanol and hexane[37,38], while other findings show that methanol produces a greater proportion than ethanol and water[39] so that we used the methanol in the initial extraction.

Overall, the results of the bioassay test showed that three extract types of *D. elliptica* are effective compounds because they had high toxicity against *Ae. aegypti* larvae. Previous studies categorized the effectiveness of plant extract larvicides into four levels, namely less effective (LC_{50}>750 mg LG^1), effective (LC_{50} = 100-750 mg LG^1), moderate (LC_{50} = 50-100 mg LG^1) and high (LC_{50}<50 mg LG^1) larvicidal activity[22]. In this study, three types of extracts namely n-hexane, methanol and ethyl acetate have high larvicidal toxicity, so that further research and development become a technical grade of larvicide are still underway. The mortality rate of *Ae. aegypti* larvae in the bioassay test were caused by the exposure of *D. elliptica* root extracts. It is proven that there are no dead larvae in the negative control (aquadest)

and 100% of larvae die in positive control. Based on the WHO standard procedure, if larval mortality in the control group was less than 20%, the results of the bioassay test can still be accepted after being corrected with Abbott's formula[36].

The n-hexane extract type causes the highest mortality rate of *Ae. aegypti* larvae compared with the other extract types since the beginning of the exposure time and progressively continues to increase for up to 24 h. The 97.6% mortality rate of *Ae. aegypti* larvae were achieved at a concentration of 10 mg LG^{1}. The toxicity of n-hexane extract is related to secondary lipophilic metabolites contained in this extract type[38]. Phytochemical screening shows that there are nine secondary metabolites found in n-hexane extract types. This extract type has never been used in the previous study in the context of the larvicidal activity test of *D. elliptica* against *Ae. aegypti* larvae. Although the toxicity of n-hexane extract is lower than petroleum ether extracts[24], this solvent has resulted in a promising extract.

Larvicidal toxicity test of ethyl acetate extract of *D. elliptica* root against *Ae. aegypti* larvae have not been performed yet. A study explored several classes of chemical compounds from *D. elliptica*, which are bound by ethyl acetate solvents namely alkaloids, flavonoids, sterols, tannins and triterpenoids[40] and tested for antimicrobial activity. The FTIR analysis of this study indicated the same lead compounds between n-hexane extract and ethyl acetate.

The methanol extract in this study showed high larvicidal toxicity with LC_{50} which was almost equivalent to previous findings in Thailand[22] but lower than findings in India[24]. This solvent is polar and it was used as an initial extraction so that it can bind many classes of chemical compounds to a broad polarity spectrum[35,41]. Phytochemical screening results indicate six classes of chemical compounds contained in the methanol extract.

FTIR results show that the most important differences in the classes of lead compounds in the three types of extracts are stilbenes, isoflavones and polyhydroxy acids. The toxicity of stilbenoids to mosquito larvae is determined by its lipophilic level[42], whereas lipophilic compounds are bound by hexane solvents. This result indicates that stilbenoids are important chemical compounds in n-hexane extract.

Water extracts have not indicated the larvicidal toxicity against the 3rd instar larvae of *Ae. aegypti* up to the concentration of 1,000 mg LG^{1}, whereas the other extract types have killed 100% of larvae at the concentration of 100 mg LG^{1}. The possible reasons are the complexity or low levels of chemical compounds bound by this universal solvent. If the bound compounds are very complex and contain many types of compounds, there is a possibility of an antagonistic mechanism between the compounds[43,44]. The second possible reason is that the water extract resulted from the last fraction so that the extract contains only a few remaining compounds, both types and levels. Although water is the universal extraction solvent, this extract only contains the polar chemical compounds bound to methanol and can be bound by water, at a low level[45]. Both of these conditions are still unclear and interesting for further study.

In the context of exploration and testing of the larvicidal activity of *D. elliptica* extracts against *Ae. aegypti* larvae in the last two decades, at least six solvents have been used by researchers, namely petroleum ether, methanol, ethanol and a combination of methanol: chloroform and methyl-chloride: methanol[22-26]. The bioassay test of these extract types showed various results. Petroleum ether extract provides a different effect based on the geographical origin of plant habitat where in Thailand shows LC_{50} of 11.17 mg LG^{1}, whereas in India 0.616 mg LG^{1}. The larvicide toxicity test of methanol extract was carried out in Thailand and Indonesia (in this study) with equivalent results respectively 13.17 and 14.066 mg LG^{1}. This result is due to differences in levels of secondary metabolites of *D. elliptica* among regions[24,29-32]. The toxicity of plant extract from a combination of methanol-chloroform solvent was more effective than methyl chloride-methanol with LC_{50} of 4.21 and 24-32 mg LG^{1}, respectively. This condition showed that the solvent types affected the dissolved secondary metabolites[28].

Exploration of various bioactive larvicidal ingredients from the roots of *D. elliptica* is an important effort in the context of Dengue vector control considering that the temephos resistance of *Ae. aegypti* larvae are increasingly widespread and have been reported in Dengue endemic areas[6-8]. Based on these conditions, community attention has increased on the natural insecticides and larvicides compounds because their advantages are easily decomposed and bioaccumulation does not occur in the environment[9]. Bioassay tests on extracts from several types of plants, including *D. elliptica* have found promising results where the findings present the varied but[22,24,46] low LC_{50}. A limitation that should be noted is that the subject of this study uses the susceptible strains of *Ae.aegypti* larvae and has not included the control group of temephos-resistant larvae. Further studies are necessary to be conducted to (1) Determine the larvicidal potential of these extracts against resistant larvae, (2) Isolate and characterize the pure or specific compounds from the extract with the highest toxicity and (3) Formulate the technical grade larvicide guided with bioassay test against the 3rd instar larvae of *Ae. aegypti* both in susceptible and temephos-resistant strains.

CONCLUSION

Three of four fractions of *D. elliptica* extract have high larvicidal toxicity against the 3rd instar larvae of *Ae. aegypti*, namely n-hexane, methanol and ethyl acetate, respectively. The highest toxicity of n-hexane extract is related to the lipophilic compounds contained and stilbene is thought to play a role in this case. FTIR analysis indicated that n-hexane and ethyl acetate extract contain similar lead compounds while the stilbenes, isoflavones and polyhydroxy acid were not found in methanol and water extract.

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SIGNIFICANCE STATEMENT

This study found the different larvicidal activity in three of four types of a tuba root extract that can be beneficial for obtaining the specific chemical compounds as larvicide material for Aedes mosquitoes. This study will help the researchers to uncover critical areas of finding alternative methods for solving the resistance problems in mosquito vector control that many researchers are unable to explore. This finding reinforces that new theories on herbal chemical compounds can be arrived at the near times.

REFERENCES

1. Kraemer, M.U., M.E. Sinka, K.A. Duda, A.Q. Mylne and F.M. Shearer *et al*., 2015. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLIFE, Vol. 4. 10.7554/eLife.08347
2. WHO, 2019. Dengue and severe dengue. Geneva, Switzerland.
3. Perez, D., P. Lefevre, L. Sanchez, L.M. Sanchez, M. Boelaert, G. Kouri and P. Van Der Stuyft, 2007. Community participation in *Aedes aegypti* control: A sociological perspective on five years of research in the health area ‘‘26 de Julio’’, Havana, Cuba. Trop. Med. Int. Health, 12: 664-672.
4. Sayono, S., W. Widoyono, D. Sumanto and R. Rokhani, 2019. Impact of dengue surveillance workers on community participation and satisfaction of dengue virus control measures in semarang municipality, Indonesia: A policy breakthrough in public health action. Osong Public Health Res. Perspect., 10: 376-384.
5. George, L., A. Lenhart, J. Toledo, A. Lazaro and W.W. Han *et al*., 2015. Community-effectiveness of temephos for dengue vector control: A systematic literature review. PLoS Negl. Trop. Dis., 10.1371/journal.pntd.0004006
6. Moyes, C.L., J. Vontas, A.J. Martins, L.C. Ng and S.Y. Koou *et al*., 2017. Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. PLoS Negl. Trop. Dis., 10.1371/journal.pntd.0005625
7. Mulyatno, K.C., A. Yamanaka, Ngadino and E. Konishi, 2012. Resistance of *Aedes aegypti* (Lin.) larvae to temephos in Surabaya, Indonesia. Southeast Asian J. Trop. Med. Public Health, 43: 29-33.
8. Ikawati, B., Sunaryo and B.F. Wahyudi, 2017. *Aedes aegypti* resistance to temephos in central java, Indonesia. Adv. Sci. Lett., 23: 3544-3546.
9. Arnason, J.T., S.R. Sims and I.M. Scott, 2012. Natural products from plants as insecticides. In: Phytoc-hemistry and Pharmacognosy, Pezzuto, J.M. and M. Kato (Eds.). Oxford, UK,.
10. Starr, F., K. Starr and L. Loope, 2003. *Derris elliptica*: poison vine, fabaceae. 1-4.
11. Orwa, C., A. Mutua, R. Kindt, R. Jamnadass and A. Simons, 2009. Agroforestry database: A tree reference and selection guide version 4.0. World Agroforestry Centre, Nairobi, Kenya.
12. Sirichamorn, Y., F.A.C.B. Adema, B. Gravendeel and P.C. Van Welzen, 2012. Phylogeny of palaeotropic *Derris*-like taxa (Fabaceae) based on chloroplast and nuclear DNA sequences shows reorganization of (infra)generic classifications is needed. Am. J. Bot., 99: 1793-1808.
13. Morel, S., J.J. Helesbeux, D. Seraphin, J. Gato and M.C. Aumond *et al*., 2012. Anti-AGEs and antiparasitic activity of an original prenylated isoflavonoid and flavonone isolated from *Derris ferruginea*. Phytochem. Lett., 6: 498-503.
14. Wu, X., Z. Song, H. Xu, H. Zhang, W. Chen and H. Liu, 2012. Chemical constituents of the aerial part of *Derris elliptica*. Fitoterapia, 83: 723-736.
15. Musa, W., J. Ahmad and C.J. Lamangantjo, 2016. Bioactive compounds in tombili seeds and tubile roots as the alternative for synthetic pesticide to protect wheats from insects and pests. Int. J. Chem. Tech. Res., 9: 604-615.
16. Lu, H.Y., L. Jing-Yu, Y. Ping and Q. Wei, 2008. Isoflavones and their derivatives from the root of *Derris elliptica* (Roxb.) Benth. Chin. J. Nat. Med., 6: 265-267.
17. Russell, D.A., W.J.S. Fong, D.G. Twigg, H.F. Sore and D.R. Spring, 2017. Stereocontrolled semisyntheses of elliptone and 12a$-Hydroxyelliptone. J. Nat. Prod., 80: 2751-2755.
18. Seiferle, E.J. and D.E.H. Frear, 1948. Insecticides derived from plants. Ind. Eng. Chem., 40: 683-691.

19. Gupta, R.C., 2007. Rotenone. In: Veterinary Toxicology: Basic and Clinical Principles, Gupta, R.C., Elsevier, Amsterdam, Netherlands, pp: 499-501.
20. Yoon, A.S., 2009. Extraction and formulation development of *Derris elliptica* for insect pest control. Ph.D Thesis, Prince of Songkla University, Thailand.
21. Zubairi, S.I., M.R. Sarmidi and R.A. Aziz, 2014. Bio-active constituents of rotenoids resin extracted from *Derris elliptica* roots: Comparison between local plant extract and SAPHYR (France) cube resin. Adv. Environ. Biol., 8: 904-909.
22. Komalamisra, N., Y. Trongtokit, Y. Rongsriyam and C. Apiwathnasorn, 2005. Screening for larvicidal activity in some Thai plants against four mosquito vector species. Southeast Asian J. Trop. Med. Public Health, 36: 1412-1422.
23. Zubairi, S.I., M.R. Sarmidi and R.A. Aziz, 2015. A preliminary study on mosquito larvicidal efficacy of rotenone extracted from Malaysia *Derris* sp. J. Teknol., 76: 275-279.
24. Dohutia, C., D.R. Bhattacharyya, S.K. Sharma, P.K. Mohapatra and K. Bhattacharjee *et al*., 2015. Larvicidal activity of few select indigenous plants of North East India against disease vector mosquitoes (Diptera: Culicidae). Trop. Biomed., 32: 17-23.
25. Sayono, U., Nurullita and M. Suryani, 2010. The effect of flavonoid concentration in tuba (*Derris eliptica*) extract on death of *Aedes aegypti* larva. J. Health Dear. Indonesia, 6: 38-47.
26. Komansilan, A., N.W. Suriani and H.T. Lawalata, 2017. Test toxic tuba root extract as a natural insecticide on larvae of *Aedes aegypti* mosquito vector of dengue fever. Int. J. Chem. Tech. Res., 10: 522-528.
27. Sayono, A. Permatasari and D. Sumanto, 2019. The effectiveness of *Derris elliptica* (Wall.) benth root extract gainst temephos-resistant *Aedes aegypti* larvae. IOP Conf. Ser.: Earth Environ. Sci., 10.1088/1755-1315/292/1/012052
28. Nguyen, A. and Y. Phan, 2014. Preliminary phytochemical analysis of different solvent extracts of *Derris elliptica* (Roxb.) Benth leaves. Int. J. Innovation Appl. Res., 2: 74-76.
29. Karahan, F., C. Avsar, I.I. Ozyigit and I. Berber, 2016. Antimicrobial and antioxidant activities of medicinal plant *Glycyrrhiza glabra* var. *glandulifera* from different habitats. Biotechnol. Biotechnol. Equip., 30: 797-804.
30. Kumar, S., A. Yadav, M. Yadav and J.P. Yadav, 2017. Effect of climate change on phytochemical diversity, total phenolic content and *in vitro* antioxidant activity of *Aloe vera* (L.) Burm. f. BMC Res. Notes, Vol. 10. 10.1186/s13104-017-2385-3
31. Sampaio, B.L. and F.B. Da Costa, 2018. Influence of abiotic environmental factors on the main constituents of the volatile oils of *Tithonia diversifolia*. Rev. Bras. Farmacogn., 28: 135-144.
32. Qi, M., X. Hua, X. Peng, X. Yan and J. Lin, 2018. Comparison of chemical composition in the buds of *Aralia elata* from different geographical origins of China. R. Soc. Open Sci., Vol.5. 10.1098/rsos.180676
33. Li, J.X., T. Hareyama, Y. Tezuka, Y. Zhang, T. Miyahara and S. Kadota, 2005. Five new oleanolic acid glycosides from *Achyranthes bidentata* with inhibitory activity on osteoclast formation. Planta. Med., 71: 673-679.
34. Koehn, F.E. and G.T. Carter, 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov., 4: 206-220.
35. Liu, Z., 2008. Preparation of botanical samples for biomedical research. Endocr. Metab. Immune Disord. Drug Targets, 8: 112-121.
36. WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides, Geneva, Switzerland. 7-12.
37. Razak, M.F.B.A., P.K. Yong, Z.M. Shah, L.C. Abdullah, S.S. Yee and I.T.C.S. Yaw, 2012. The effect of varying solvent polarity on extraction yield of *Orthosiphon stamineus* leaves. J. Appl. Sci., 12: 1207-1210.
38. Abarca-Vargas, R., C.P. Malacara and V. Petricevich, 2016. Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougainvillea×buttiana* holttum and standl, (var. Rose) extracts. Antioxidants, 10.3390/antiox5040045
39. Borah, R. and S.P. Biwas, 2018. Tulsi (*Ocimum sanctum*), excellent source of phytochemicals. Int. J. Environ. Agric. Biotech., 3: 1732-1738.
40. Khan, M.R., A.D. Omoloso and Y. Barewai, 2006. Antimicrobial activity of the *Derris elliptica*, *Derris indica* and *Derris trifoliata* extractives. Fitoterapia, 77: 327-330.
41. Tiwari, P., B. Kumar, M. Kaur, G. Kaur and H. Kaur, 2011. Phytochemical screening and extraction: A review. Int. Pharm. Sci., 1: 98-106.
42. Sobolev, V.S., S.I. Khan, N. Tabanca, D.E. Wedge and S.P. Manly *et al*., 2011. Biological activity of peanut (*Arachis hypogaea*) phytoalexins and selected natural and synthetic stilbenoids. J. Agric. Food Chem., 59: 1673-1682.
43. Milugo, T.K., L.K. Omosa, J.O. Ochanda, B.O. Owuor, F.A. Wamunyokoli, J.O. Oyugi and J.W. Ochieng, 2013. Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (*Rauvolfia caffra* sond.): Further evidence to support biotechnology in traditional medicinal plants. BMC Complement. Altern. Med., Vol. 13. 10.1186/1472-6882-13-285
44. Celep, E., Y. Inan, S. Akyuz and E. Yesilada, 2017. The bioaccessible phenolic profile and antioxidant potential of *Hypericum perfoliatum* L. after simulated human digestion. Industrial Crops Prod., 109: 717-723.
45. Dhawan, D. and J. Gupta, 2017. Comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. Int. J. Biol. Chem., 11: 17-22.
46. Scalvenzi, L., M. Radice, L. Toma, F. Severini and D. Boccolini *et al*., 2019. Larvicidal activity of *Ocimum campechianum*, *Ocotea quixos* and *Piper aduncum* essential oils against *Aedes aegypti*. Parasite, 10.1051/parasite/2019024