

# Larvacidal activity evaluation of the chemical compound isolated from n-hexane extract of Derris elliptica root againsts the Temepos-susceptible strain of Aedes aegypti larvae

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## Larvicidal activity evaluation of the chemical compounds isolated from n-hexane extract of *Derris elliptica* root against the Temephos-susceptible strain of *Aedes aegypti* larvae

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**Abstract.** Sayono S, Anwar R, Sumanto D. 2022. Larvicidal activity evaluation of the chemical compounds isolated from n-hexane extract of *Derris elliptica* root against the Temephos-susceptible strain of *Aedes aegypti* larvae. *Biodiversitas* 23: 757-764. *Aedes aegypti* is the main vector of Dengue, Chikungunya, and Zika diseases. Temephos resistance of this species has hampered vector control efforts worldwide. Studies proved that *Derris elliptica* extracts effectively controlled *Aedes* larvae, so the active compound needed to be isolated. This study evaluated the larvicidal activity of the chemical compounds isolated from n-hexane fractions of Tuba roots against the temephos-susceptible *Ae. aegypti* larvae. Six isolates were obtained from three of the seven n-hexane fractions. Three levels of concentration of each isolate were formulated for a preliminary bioassay test, and resulted in the two most active compounds, isolates 3 and 6. The final bioassay test results indicated that isolate 3 was more active than isolate 6 with LC<sub>50</sub> and LC<sub>90</sub> after 24 hours of exposure were 1.607 and 7.399 ppm, and after 33 hours of exposure were 0.926 and 3.206 ppm respectively. Isolate 6 also had high larvicidal activity after 48 hours of exposure, with LC<sub>50</sub> and LC<sub>90</sub> were 1.056 and 4.647 ppm. Further studies need to be carried out to determine the chemical structures and toxicity mechanisms of the bioactive compounds.

**Keywords:** *Aedes aegypti* larvae, chemical isolates, *Derris elliptica*, larvicidal activity, n-hexane fraction, Temephos-susceptible strain

### INTRODUCTION

*Aedes aegypti* is the principle vector of human viral diseases including Dengue, Chikungunya, Yellow fever, and Zika (Wibowo et al. 2010; Powell et al. 2018). Since these arboviral diseases have become a threat and a global public health problem (Marchi et al. 2018; Chaiphongpachara et al. 2019; Girard et al. 2020), community attention to the species increased according to the escalation and expansion of the disease occurrence from Africa to other regions worldwide (Weetman et al. 2018). In Dengue endemic areas, efforts to control this mosquito species have become a priority since there are no viral drugs and vaccines are still being developed (Arredondo-García et al. 2018; Plennevauz et al. 2018). In this case, people in endemic areas prefer to use insecticides to control these arboviral disease vectors where the organophosphate group is the most dominant (WHO 2009; Manjarres-Suarez and Alivero-Verbel 2013). The high intensity of community use with uncontrolled doses has led to resistance of *Ae. aegypti* to various classes of insecticides. This condition has spread in many countries, especially in America, Asia, and Africa (Manjarres-Suarez and Olivero-Verbel 2013).

Temephos is an active insecticide compound in the organophosphate group that is most widely used in the control of *Ae. aegypti* larvae in endemic areas of arboviruses worldwide (WHO 2009) for seven decades

(Manjarres-Suarez and Alivero-Verbel 2013), although it does not always reduce the density of the Dengue vector population. This condition is due to inconsistencies in use (George et al. 2015; Arostegui et al. 2017), low coverage of exposed water containers, especially in rural areas (Legorreta-Soberanis et al. 2017). On the other hand, long-term use of temephos with operational deficiency has led to the emergence of resistant-strains of *Ae. aegypti* to this active ingredient (Cediak et al. 2016) and has become a serious problem in controlling this arbovirus vector. To solve this problem, the researchers explored active compounds from natural materials that are biodegradable, non-persistent, and not bio-accumulative in the environment (Arnason et al. 2012).

Phytochemical screening and larvicidal activity evaluation have been carried out on various plant species, including *Derris elliptica* with varying results (Komalamisra et al. 2005). The wild plant in the agricultural farm which is commonly found in South to Southeast Asia has been traditionally used by community for a long time as a fish poison and plant pest insecticide (Starr et al. 2003; Sirichamorn et al. 2012). Studies on the larvicidal activity of various phytochemical compounds in *D. elliptica* extract against *Ae. aegypti* larvae have been reported from several countries with varying methods and results. Studies in Thailand showed that the effective doses (LC<sub>50</sub> and LC<sub>90</sub>) of the ethanol extract of *D. elliptica* against *Ae. aegypti* larvae were 20.49 and 47.49 ppm

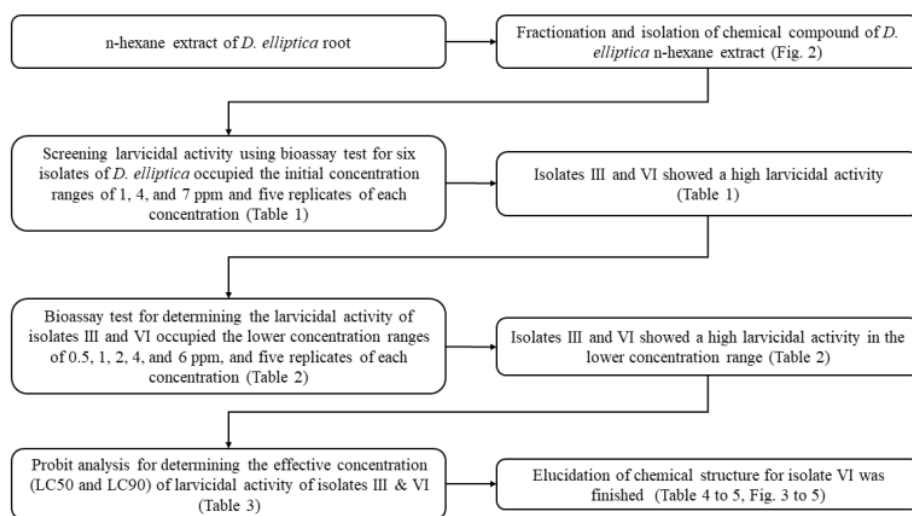
(Komalamisra et al. 2005), whereas a study in India reported the lower effective doses of petroleum ether extract (0.616 and 1.44 ppm) and methyl chloride (4.21 and 12.40 ppm) (Dohutia et al. 2015). Study with specific extraction methods shows that the solvent combinations of methyl chloride: methanol 1:1 produces an effective dose (LC<sub>50</sub>) of 24 ppm (Zubairi et al. 2015). The bioassay test of four *D. elliptica* extract fractions with different polarity, namely water, methanol, ethyl acetate, and n-hexane on *Ae. aegypti* larvae showed different larvicidal activity, and n-hexane extract was the most active extract with LC<sub>50</sub> of 4,088 ppm (Sayono et al. 2020), and isolation of specific compounds from this fraction is recommended. This study aims to evaluate the larvicidal activity of chemical compounds isolated from the n-hexane extract of *D. elliptica* root against susceptible-Temephos *Ae. aegypti* larvae.

### MATERIAL AND METHODS

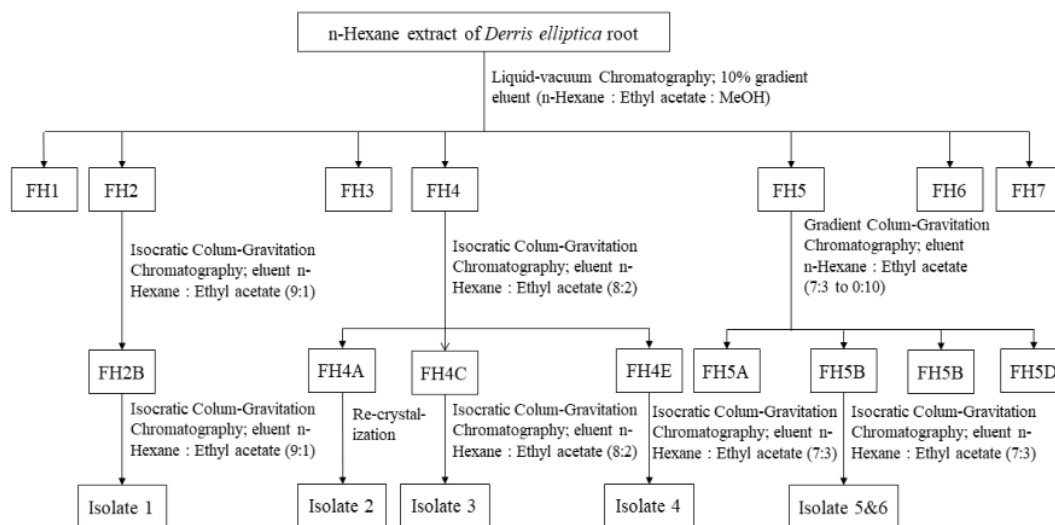
The work sequent of this study consist of seven steps, namely extract fractionation and pure chemical compound isolation, screening larvicidal potency use the initial concentration, determination bioassay test, analyzing the effective concentration, and elucidating the chemical structure of pure isolate (Figure 1). *Derris elliptica* extract used in this study is a product of previous experiment where n-hexane extract has the highest larvicidal potential among other types of extracts (Sayono et al. 2020).

### Fractionation and isolation of *Derris elliptica* roots

Fractions of *D. elliptica* were conducted by separated by using liquid-vacuum chromatography with n-hexane: ethyl acetate: methanol 10% gradient eluents, and resulted in seven grouped-fractions, namely n-hexane fraction 1 (FH1) to FH7. As much as 200 mg of FH2 was separated by using column-gravitation chromatography with n-hexane: ethyl acetate eluent (9:1) resulting in five subfractions, namely FH2A to FH2E. FH2B subfraction was purified by using column-gravitation chromatography with the same eluent resulting in as much as 30 mg of isolate 1. The separation process of FH4 (420 mg) used column-gravitation chromatography with n-hexane: ethyl acetate eluent (8:2) resulting in six subfractions of FH4A to FH4F. As much as 40 mg of FH4A subfraction was recrystallized with methanol to obtain isolate 2. FH4C subfraction (120 mg) was purified by using column-gravitation chromatography with eluent solvent of n-hexane: ethyl acetate (8:2) resulting in as much as 15 mg of isolate 3. Isolate 4 was purified from FH4E subfraction using column-gravitation chromatography with eluent solvent n-hexane: ethyl acetate (7:3). FH5 fraction was separated by using column-gravitation chromatography with eluent solvent n-hexane gradually from 7:3 to 0:10 and resulted in four subfractions, namely FH5A to FH5D. Isocratic purification of FH5B subfraction used column-gravitation chromatography with n-hexane: ethyl acetate eluent solvent (7:3) resulting in isolate 5 and 6 (Figure 2).



**Figure 1.** The consolidated report of trial. There were seven steps of the study started from fractionation and isolation of pure chemical compounds, screening bioassay use initial concentration, determination bioassay test for isolates III and VI, Probit analysis to determine the effective concentration, and elucidation of pure chemical structure of isolate VI



**Figure 2.** Fractionation and isolation of phytochemical compound from n-hexane extract of *Derris elliptica* roots. Seven fractionates and six isolates were obtained from the n-hexane fraction of *Derris elliptica*. FH1-FH7: n-hexane fraction number 1 to 7. FH2B: n-hexane fraction number 2 subfraction B, etc.

#### Collecting and rearing the *Aedes aegypti* larva

Larval surveys were conducted from January to March 2020 in Sambiroto village, Semarang City, Central Java Province, Indonesia. Morphological identification of mosquito species was carried out in the Laboratory of Epidemiology and Tropical Diseases of Public Health Faculty, Universitas Muhammadiyah Semarang, Indonesia based on the Walter Reed guideline (WRBU 2020). *Aedes aegypti* mosquitoes were reared through the fourth generation to obtain sufficient numbers with uniform age. The 3<sup>rd</sup> instar larvae were subjected to a bioassay test to evaluate the larvicidal activity of secondary metabolites of *D. elliptica* root after their susceptibility status to Temephos were determined (WHO 2016) (Table 1).

#### Bioassay tests for determining the larvicidal activity of Tuba 1-6 isolates

Initial bioassay test of this study were performed by using the previous lethal concentration 50% of n-hexane extract of *D. elliptica* (Sayono et al. 2020), namely 4.086 mg L<sup>-1</sup>. Based on the LC50, a new concentration range of 1, 4, and 7 mg L<sup>-1</sup> was set and occupied for the six isolates (Table 2). Five experiment replicates were involved in each concentration level, and each replicate contains twenty third instar larvae of *Ae. aegypti*. The results of the initial bioassay test were used to determine the new lower concentration ranges at the next testing steps until the lowest effective concentrations (LC<sub>50</sub> and LC<sub>90</sub>) are obtained. The final concentration ranges of tuba isolates were 0.5, 1, 2, 4, and 6 ppm. The larvicidal bioassay test experiment was accompanied by a positive control (Temephos 0.02 ppm) and a negative control (aquadest).

**Table 1.** Results of initial bioassay test of the pure compound isolated from n-hexane fraction of *Derris elliptica* extract against *Aedes aegypti* larvae

Isolates	Dosages (ppm)	Larval mortality rate (%)	
		24 hrs	48 hrs
I	1	0	-
	4	0	-
	7	0	-
II	1	0	-
	4	0	-
	7	0	-
III	1	45.0	-
	4	70.5	-
	7	92.5	-
IV	1	5.0	22.5
	4	5.0	47.5
	7	7.5	57.5
V	1	5.0	45.0
	4	7.5	60.0
	7	10.0	60.0
VI	1	20.0	57.5
	4	30.0	80.0
	7	65.0	92.5
Positive control (Temephos)	0.02	100	100
Negative control (Aquadest)	-	0	0

**Table 2.** Results of larvicidal activity determination of pure compound isolates number III and VI of *Derris elliptica* against *Aedes aegypti* larvae using the final concentration ranges of 0.5, 1, 2, 4, and 6 mg L<sup>-1</sup>

Isolates	Dosages (mg L <sup>-1</sup> )	Larval mortality rate (%)	
		24 hrs	48 hrs
III	0.5	17.5	27.5
	1	30.0	45.0
	2	65.0	77.5
	4	72.5	92.5
	6	90.0	100.0
VI	0.5	5.00	25.0
	1	26.3	51.3
	2	41.3	61.3
	4	46.3	71.3
	6	75.0	100.0
Positive control (Temephos)	0.02	100	100
Negative control (Aquadest)	-	0	0

### Elucidation of chemical compound

This step was carried out to determine the structure of chemical compounds from tuba root isolates which had high larvicidal activity. There were two isolates with the highest larvicidal activity, namely isolates III and VI, but only isolate VI had completed structural elucidation. The process of elucidating the chemical structure of isolate VI combines two methods, namely spectroscopy and Nuclear Magnetic Resonance (NMR). Spectroscopy uses ultraviolet (UV) light with a 200-400 nm wavelength and infrared (IR). UV spectroscopy was used to identify double bonds and aromatic conjugates, while IR was used to identify functional groups. NMR of one dimension (<sup>13</sup>C-NMR, <sup>1</sup>H-NMR dan DEPT 135°) and two dimensions (*Heteronuclear Multiple Quantum Coherence* [HMQC], *Heteronuclear Multiple Bond Connectivity* [HMBC] dan <sup>1</sup>H-<sup>1</sup>H COSY [*Correlation Spectroscopy*]) were used to understand the number, kind, and environment of carbon and proton. The spectrophotometer FTIR Perkin Elmer Spectrum 1900, JEOL JNM A-500 nuclear magnetic resonance (NMR) spectrometer, and TMS as internal standard and chemical shift (δ) in ppm units were used.

### Data analysis

Larvicidal activity is indicated with the average of larval mortality of *Ae. aegypti* larvae. This variable was analyzed based on the five levels of isolates concentration, five replicates of each concentration level, and two different of observation times (24 and 48 hours) by using ANOVA test. The result was followed by Probit test to analyze the effective concentration indicating by LC<sub>25</sub>, LC<sub>50</sub>, and LC<sub>90</sub> percent. All data analysis was performed by using SPSS 16.0 version.

### Ethical consideration

The protocol of this study was reviewed by the Ethic Committee of Health Research of Public Health Faculty of Universitas Muhammadiyah Semarang with registration number 231/KEPK-FKM/UNIMUS/2019.

## RESULTS AND DISCUSSION

### *Derris elliptica* root extract, fractionation, and pure compound isolation

This study is a part of the exploration of phytochemicals with larvicidal potential and focuses on the *D. elliptica* or tuba root. This plant is interesting for further investigation because of several aspects: (i) its toxicity has been used by traditional communities as fish poison and insecticide for plant pests (Starr et al. 2003); (ii) the potential for larvicide varies widely based on geography and the screening method applied (Komalamisra et al. 2005; Dohutia et al. 2015; Zubairi et al. 2015; Sayono et al. 2020), and (iii) agricultural weeds that grow abundantly. *Derris elliptica* is a vine both horizontally on the ground and wrapped around and covering towering trees commonly found in South to Southeast Asia, and even spread to Africa and America. These plants are invasive to moderate to high levels and grow rapidly in tropical climates (CABI 2020). Utilization of this plant has a positive and strategic impact from a health and environmental aspects, especially the promising potential of larvicides and eradicating weeds.

This study applied a tiered or serial non-parallel screening method guided bioassay test to evaluate the larvicidal potential of outcomes at each stage. This is intended to evaluate the larvicidal potential of each type of extract produced. The screening process starts from the extraction of the polar tuba root compound with methanol solvent, and then the polar methanol extract is partitioned with non-polar n-hexane to bind the non-polar compounds, leaving other parts in the water solution. The other part was partitioned with ethyl acetate to bind the semi-polar compound, leaving an aqueous extract.

There are seven fractions resulted from this study, and eight subfraction were identified from the stronger fraction, namely FH2, FH4, and FH5. Six isolates of pure chemical compounds were resulted from these subfractions. Visual characteristics showed that isolates 1, 2, and 4 are transparent (colorless) crystals while isolates 3, 5, and 6 are yellow crystals. This study reached a new step in exploration of chemical compounds of *D. elliptica* and their potency.

### Bioassay test

Initial bioassay test showed that there were two chemical compound isolates of *D. elliptica* root have a high larvicidal potency, namely isolate III and VI. Isolates IV and V showed a low larvicidal activity while isolates I and II did not result the larval mortality. It meant that only isolates III and VI contain an active ingredient. Based on the concentration range of 1, 4, and 7 ppm for 24 hours, isolate 1 and 2 did not indicate the larvicidal activity. There is no dead *Ae. aegypti* larvae were found after exposure to the compounds. The six isolates of chemical compounds were produced from a combination of chromatography and purification methods (Ingle et al. 2017), which are visually differentiated into four secondary metabolite groups, and only the flavonoid group that has high larvicidal potential. These findings indicate that the larvicide potency of n-hexane extract is influenced by the flavonoid content. This is shown by the difference in the larvicidal effect of isolates

1, 2, and 4 (non-flavonoids) with isolates 3 and 6 (flavonoid group) which have three times more potency than in the form of extracts (Sayono et al. 2020; Zubairi et al. 2015; Komalamisra et al. 2005), although slightly lower than petroleum ether extract and equivalent to methyl chloride extract (Dohutia et al. 2015). The highest larvicidal activity was found in isolate 3 with a mortality rate of 45-92.5%, followed by isolate 6 (20-60%) and isolates 4 and 5 (< 10%). The mortality rate of isolate 4, 5, and 6 increased after 48 hours of exposure namely 22.5-57.5%, 45-60.0%, and 45-92.5% respectively. Based on the results, isolates 3 and 6 were used to determine the effective larvicidal activity by the next step of the bioassay test with the lower concentration range of 0.5, 1, 2, 4, and 6 ppm.

### Fig 3 bioassay test and the effective concentration

The final result of bioassay test showed that the larvicidal activity of isolate 3 better than isolate 6 (Table 3). Exposure to isolate 3 for 24 hours has caused mortality rates for *Ae. aegypti* larvae of 17.5-90% with LC<sub>50</sub> and LC<sub>90</sub> were 1.607 (1.250-2.025) and 7.399 (5.147-13.284) ppm, while exposure to isolate 6 caused mortality rates of 5-75% with LC<sub>50</sub> and LC<sub>90</sub> were 2.509 (2.098-3.048) and 13.894 (9.602-24.084) ppm respectively. However, both isolates 3 and 6 had good larvicidal activity after 48 hours of exposure where the mortality rate of isolate 3 ranged from 27.5-97.5% with LC<sub>50</sub> and LC<sub>90</sub> were 0.926 (0.714-1.143) and 3.206 (2.459-4.782) ppm, and mortality rate of isolate 6 ranged from 25-100% with LC<sub>50</sub> and LC<sub>90</sub> were 1.056 (0.868-1.249) and 4.647 (3.661-6.459) ppm, respectively (Table 4). These findings indicated that the larvicidal activity of isolate 6 was slightly lower and slower than isolate 3. Three solvents produced extracts and isolates with high larvicidal potential, namely petroleum ether, n-hexane, and methyl chloride. Flavonoids include more than 4,000 specific compounds which are grouped into flavonols, flavones, flavanones, isoflavones, and anthocyanidins (Paula-Ribeiro-Povinelli et al. 2019). The main target site for flavonoid compounds is Acetylcholinesterase where the compound works to inhibit the activity of this enzyme (Perumalsamy et al. 2015). These compounds also disrupt the endocrine and hormonal systems (Ge et al. 2015) and reducing the esterase and monoxygenase enzymes (Visetson et al. 2001).

### Chemical structure of isolate VI

UV spectroscopy in 200-400 nm wavelength identifies the double-bond and aromatic conjugation (Supratman 2010). The results (Figure 3.A) show the presence of a 315-

350 nm peak which is a conjugated carbonyl (C=CC=O) with an electron transition of n→π\* and a 245-265 nm peak with an electron transition of →π\*, indicating the existence of a conjugated double bond (C=C-C=C) (Mabry et al. 1975). Figure 3.B showed the presence of typical absorptions such as free-OH groups at max 3,452 cm<sup>-1</sup>, C-H stretching vibrations at max 2,938 cm<sup>-1</sup>, chelated α,β-unsaturated carbonyl groups bonded to aromatics at max 1,674, 1,607, and 1,509 cm<sup>-1</sup>, the stretching vibration of C-O at max 1,262 cm<sup>-1</sup> and the methoxy group at max 1,088 cm<sup>-1</sup> (Pavia et al. 2008). Interpretation of IR spectrum of Tuba VI isolate showed in Table 4.

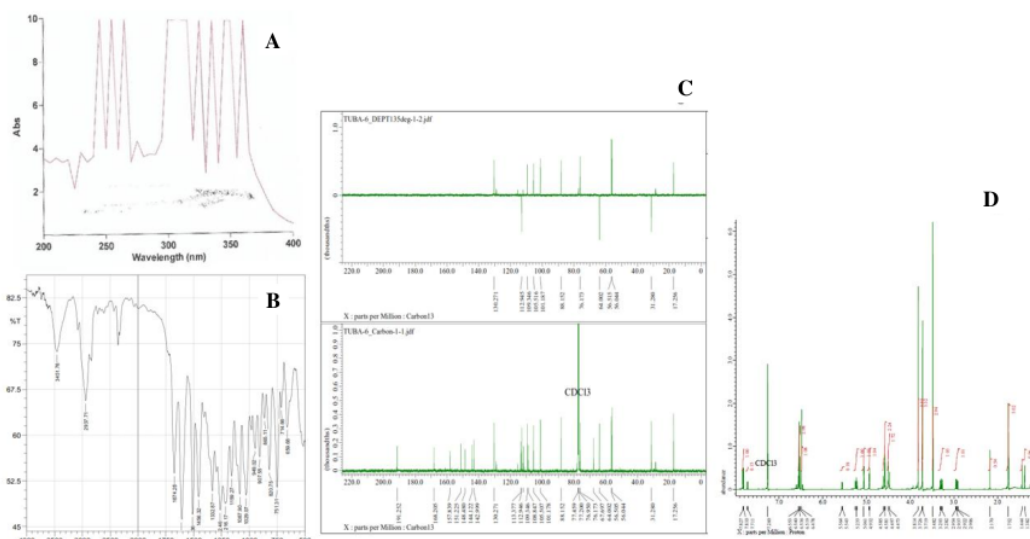
The spectrum of the <sup>13</sup>C-NMR showed the presence of twenty-three carbon signals consisting of one carbonyl signal at δC 191.3 ppm, five oxygenated aromatic carbon signals at δC 168.2; 157.8; 151.2; 148.5; and 144.1 ppm, one oxygenated methylene carbon signal at δC 67.7 ppm, two methylene carbon signals at δC 31.3 and 112.9 ppm, two oxygenated methine signals at δC 88.2 and 76.2 ppm, one signal oxygenated quaternary carbon at δC 64.0 ppm, four methine signals at δC 130.3; 109.3; 105.5; and 101.2 ppm, two methoxy signals at δC 56.5 and 56.0 ppm, one methyl at δC 17.3 ppm and six quaternary carbon signals at δC 143.0; 113.4; 111.9; and 108.8 ppm. DEPT 135° analysis showed that the methoxy and methoxy carbons were the top peaks (positive) while the curtener carbon did not appear as the peak (Figure 3.C). The <sup>1</sup>H-NMR spectrum showed a shift at δH 7.82 ppm (1H, d, J = 8.5 Hz); 6.54 (1H, s); 6.52 (1H, s); 6.48 (1H, s); 5.23 (1H, t, J = 9 Hz) and 4.59 (1H, m) indicated the presence of six methines. Then there is a shift in δH 4.93 & 5.06 (2H, s); 4.49 (2H, m, J = 12 Hz); and 2.93 & 3.29 (2H, q, J = 7.75 & 15.5 Hz) indicated the presence of three methylene, shifts of δH 3.81 (3H, s) and 3.72 (3H, s) indicated the presence of two methoxy and δH shift of 1.75 ppm (3H, s) indicated the presence of methyl (Figure 3.D).

Table 4. Interpretation of IR spectrum of TUBA-6 compound

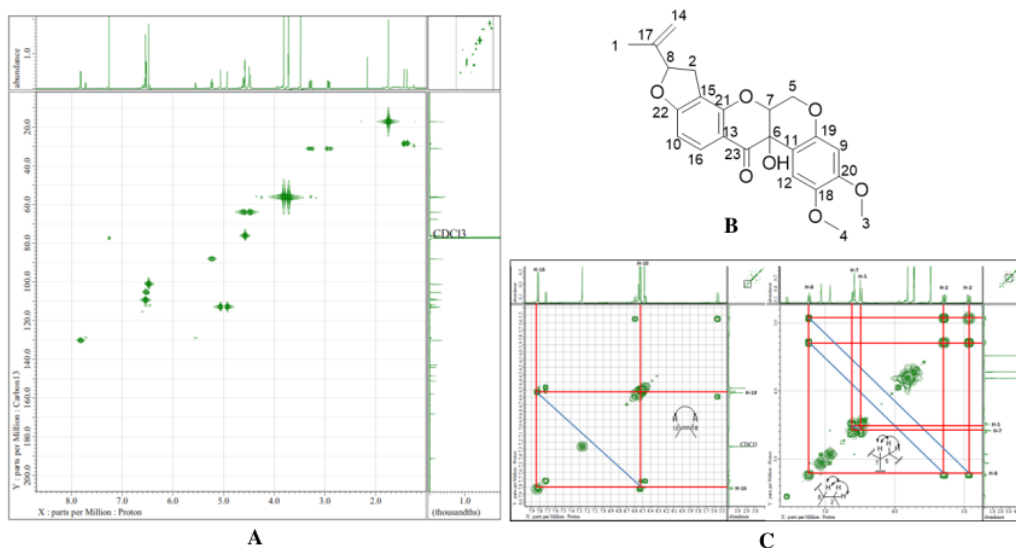
ν <sub>max</sub> / cm <sup>-1</sup>	Band shape	Intensity	Prediction
3452	Sharp	Low	Free OH
2938	Sharp	Medium	C-H stretch
1674	Sharp	High	C=O stretch
1607	Sharp	High	C=C stretch
1509	Sharp	High	C=C stretch
1088	Sharp	High	Oxygenized-methyl

Table 3. Results of Probit analysis showed the LC<sub>50</sub> and LC<sub>90</sub> of isolates III and VI of *Derris elliptica* against *Aedes aegypti* larvae based on final concentration ranges of 0.5, 1, 2, 4, and 6 mg L<sup>-1</sup>

Isolates	Exposure time (hours)	Regression equation	Lethal Concentration (ppm)		Chi-Square	p-value
			LC50 (95% Confidence limits)	LC90 (95% Confidence limits)		
III	24	Y = -0.398+1.932X	1.607 (1.250-2.025)	7.399 (5.147-13.284)	6.539	0.587
	48	Y = 0.079+2.377X	0.926(0.714-1.143)	3.206(1.459-4.782)	7.594	0.474
VI	24	Y = -0.689+1.724X	2.509 (2.098-3.048)	13.894 (9.602-24.084)	12.948	0.795
	48	Y = -0.047+1.992X	1.056 (0.868-1.249)	4.647 (3.661-6.459)	16.865	0.532



**Figure 3.** The spectrum results of spectroscopy and NMR: (A) UV and (B) IR spectroscopy; (C)  $^{13}\text{C}$ -NMR and DEPT 135° (500MHz,  $\text{CDCl}_3$ ); and (d)  $^1\text{H}$ -NMR (500 MHz,  $\text{CHCl}_3$ ) of Tuba-VI pure chemical compound isolate



**Figure 4.** The HMQC spectrum (A), prediction chemical structure (B), and  $^1\text{H}$ - $^1\text{H}$  COSY (C)

The HMQC spectrum showed the six proton correlations with carbon atoms one bond apart (bond to each other). The correlation between H-1 at  $\delta\text{H}$  1.75 ppm (3H, s) and C-1 at  $\delta\text{C}$  17.3 ppm indicate the presence of methyl carbon. Correlation of H-2 at  $\delta\text{H}$  2.93 & 3.29 ppm (2H, q,  $J = 7.75$  & 15.5 Hz) with C-2 at  $\delta\text{C}$  31.3 ppm; H-5 at  $\delta\text{H}$  4.49 ppm (2H, m,  $J = 12$  Hz) with C-5 at  $\delta\text{C}$  64.0 ppm and H-14 at  $\delta\text{H}$  4.93 & 5.06 ppm (2H, s) with C-14 at  $\delta\text{C}$  112.9 ppm confirmed that C-2, C-5, and C-4 were methylene carbon. Correlation of H-3 at  $\delta\text{H}$  3.81 ppm (3H,

6 s) with C-3 at  $\delta\text{C}$  56.0 ppm and H-4 at  $\delta\text{H}$  3.72 ppm (3H, s) with C-4 at  $\delta\text{C}$  56.5 ppm showed carbon methoxy and correlation of H-7 at  $\delta\text{H}$  4.59 ppm (1H, m) with C-7 at  $\delta\text{C}$  76.2 ppm; H-8 at  $\delta\text{H}$  5.23 ppm (1H, t,  $J = 9$  Hz) with C-8 at  $\delta\text{C}$  88.2 ppm; H-9 at  $\delta\text{H}$  6.48 ppm (1H, s) with C-9 at  $\delta\text{C}$  101.2 ppm; H-10 at  $\delta\text{H}$  6.52 ppm (1H, s) with C-10 at  $\delta\text{C}$  105.5 ppm; H-12 at  $\delta\text{H}$  6.54 ppm (1H, s) with C-12 at  $\delta\text{C}$  109.3 ppm; and H-16 at  $\delta\text{H}$  7.82 ppm (1H, d,  $J = 8.5$  Hz) with C-16 at  $\delta\text{C}$  130.3 ppm indicating the presence of proton methine (Figure 4.A). One-dimensional NMR spectroscopic

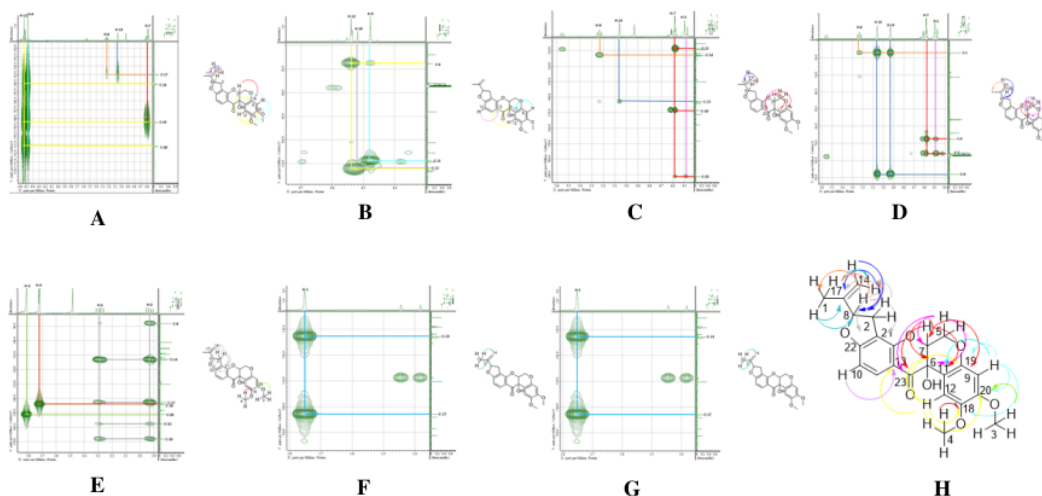
analysis of the Tuba VI compound in  $\text{CHCl}_3$  solvent obtained the data in Table 5 and it is suspected that this compound is a group of rotenoid compounds with an OH group at the C-6 position (Figure 4.B). The  $^1\text{H}$ - $^1\text{H}$  COSY analysis was used to determine the proton-to-proton correlation between three bonds. This correlation is indicated by the presence of a cross peak. The COSY  $^1\text{H}$ - $^1\text{H}$  spectrum showed a correlation between H-10 at  $\delta\text{H}$  6.52 ppm (1H, s) and H-8 at  $\delta\text{H}$  5.23 ppm (1H, t,  $J = 9$  Hz) with H-2 at  $\delta\text{H}$  2.93 & 3.29 ppm (2H, q,  $J = 7.75$  & 15.5 Hz), and  $\delta\text{H}$ -7 at H 4.59 ppm (2H, m) with H-5 at  $\delta\text{H}$  4.49 (2H, m,  $J = 12$  Hz) so it can be concluded that C-10 at  $\delta\text{C}$  105.5 ppm coexists with C-16 at  $\delta\text{C}$  130.3 ppm, C-8 at  $\delta\text{C}$  88.2 ppm coexists with C-2 at  $\delta\text{C}$  31.3 ppm, and C-7 at  $\delta\text{C}$  76.2 ppm coexists with C-5 at  $\delta\text{C}$  64.0 ppm with one bond distance (Figure 4. C).

The HMBC spectrum was used to determine the proton-to-carbon correlation between two to three bonds, confirming that Tuba VI is a rotenoid compound (Figure 5.A-G). The first correlation is H-7 which correlates  $^1J$  with C-7,  $^2J$  with C-11 and C-6,  $^3J$  with C-23, and  $^4J$  with C-19. Furthermore, H-14 which has a correlation of  $^2J$  with C-17 and  $^3J$  with C-8. There is also a correlation between H-8 and C-17 as far as two bonds ( $^2J$ ), C-14 and C-1 as far as three bonds ( $^3J$ ). Then, H-9 correlated sequentially with C-19 and C-20 by two bonds ( $^2J$ ), and C-18 and C-11 by three bonds ( $^3J$ ). The protons of methine H-12 are correlated sequentially with C-18 by two bonds ( $^2J$ ) C-6, C-19 and C-20 by three bonds ( $^3J$ ) and C-13 by five bonds ( $^5J$ ) while H-10 is correlated with C-13 by 3 bonds ( $^3J$ ). The methylene protons C-5 correlated sequentially with C-7 by one bond ( $^1J$ ), C-6 and C-23 by four bonds ( $^4J$ ) while H-2 correlated sequentially with C-8 and C-21 by two bonds ( $^2J$ ), C-22 and C-17 by three bonds ( $^3J$ ) and C-14 by three bonds ( $^3J$ ). The methoxy protons H-3 and H-4 are sequentially correlated with C-20 and C-18 by three bonds ( $^3J$ ). The methyl proton H-1 is sequentially correlated with

C-17 by two bonds ( $^2J$ ), C-8 and C-14 by three bonds ( $^3J$ ). The correlation of Tuba VI compounds can be seen in Figure 5.H. Based on the analysis of one-dimensional NMR ( $^{13}\text{C}$ -NMR,  $^1\text{H}$ -NMR and DEPT 135 $^\circ$ ) and two-dimensional (HMQC, HMBC and  $^1\text{H}$ - $^1\text{H}$  COSY) compounds, Tuba VI was found in the form of 6a-hydroxy-13-dimethoxy-2-(prop-1-en-2-yl)-1,2,12,12a-tetrahydrochromeno[3,4-b]furo[2,3-h]chromen-6(6aH)-one or more commonly known as rotenolone.

**Table 5.** Interpretation of NMR 1D dan HMQC (500 MHz,  $\text{CHCl}_3$ ) Data

C position	$\delta\text{C}$ (ppm)	$\delta\text{H}$ (ppm), Mult, $J$ (Hz)	Prediction
1	17.3	1.75 (3H, s)	-Cq-CH <sub>3</sub>
2	31.3	2.93 & 3.29 (2H, q, $J = 7.75$ & 15.5 Hz)	-CH <sub>2</sub> -CH-
3	56.0	3.81 (3H, s)	-O-CH <sub>3</sub>
4	56.5	3.72 (3H, s)	-O-CH <sub>3</sub>
5	64.0	4.49 (2H, m, $J = 12$ Hz)	-CH <sub>2</sub> -CH-
6	67.7		
7	76.2	4.59 (1H, m)	-CH-CH <sub>2</sub> -
8	88.2	5.23 (1H, t, $J = 9$ Hz)	-CH-CH <sub>2</sub>
9	101.2	6.48 (1H, s)	-CH-
10	105.5	6.52 (1H, s)	-CH-
11	108.8		Cq
12	109.3	6.54 (1H, s)	-CH-Cq-
13	111.9		Cq
14	112.9	4.93 & 5.06 (2H, s)	-CH <sub>2</sub> -
15	113.4		Cq
16	130.3	7.82 (1H, d, $J = 8.5$ Hz)	-CH-CH-
17	143.0		Cq
18	144.1		-O-Cq
19	148.5		-O-Cq
20	151.2		-O-Cq
21	157.8		-O-Cq
22	168.2		-O-Cq
23	191.3		C=O



**Figure 5.** The HMBC spectrum (A-G) and correlation (H) of chemical compound of Tuba VI indicates a rotenolone



In conclusion, this study obtained six secondary metabolites isolating from n-hexane fraction of *D. elliptica* root, namely isolate 1 to 6. Two of the six isolates (Tuba III and VI) have high larvicidal activity against the Temephos-susceptible *Ae. aegypti* larvae. Elucidation of a chemical structure of isolate VI was finished and indicated a rotenolone compound. The chemical structure of isolate III, and toxication mechanisms of all active compounds are necessary conducted to prepare the technical grade of larvicide for this finding.

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