Larvicidal Activity of Ethyl Acetate Extract of Derris elliptica Root

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Original Article

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

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Abstract

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

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

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

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

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

Introduction

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

The results of monitoring of dengue vector susceptibility in Central Java, Indonesia showed a wide spread of resistance to cypermethrin 0.05 % (1), as occurs in various Dengue endemic areas in other countries (4, 9, 10). Cypermethrin is one of the active ingredients of pyrethroid class insecticide which has caused knockdown resistance (kdr) (11). This resistance mechanism was indicated with the target site insensitivity

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in the voltage-gated sodium channel (VGSC) gene (1, 12). The action mechanism of cypermethrin is different from the temephos. This compound is an active ingredient of organophosphate insecticide class which inhibited the acetylcholinesterase enzyme (13). This different mechanism of action is interesting to be studied in understanding the larvicidal activity spectrum of *D. elliptica* root extracts.

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

Materials and Methods

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

The subjects of this study were the offspring filial 2 (F2) larvae of the cypermethrin 0.05% resistant strain of *Ae. aegypti*. The parental mosquitoes were the F1 larvae of the *Ae. aegypti* that is reared from F0 larvae obtaining from a household survey in the Dengue endemic areas in the Community Health Center of Kedung Mundu, Tembalang District, Semarang City, and subjected to bioassay test using the Cypermetrhin-0.05% compound. The result of the bioassay test showed a mortality rate of 85%, which indicated that the mosquito population was resistant to the pyrethroid compound (21). Larvae were maintained in the Epidemiology and Tropical Diseases laboratory of Public Health Faculty of Universitas Muhammadiyah Semarang, Indonesia. The larvae were placed on a plastic tray containing tap water. Conditions of temperature and humidity were maintained in the range of 28±2 °C and 75±10%. The larvae were fed dog food. Bioassay tests apply the WHO standard procedures for larvicide testing (21). There are three important parts at this stage, namely preparation of extract concentration, selection of research subjects, and exposure of research subjects with various *D. elliptica* extracts. The concentration of the bioassay test used a range of 10, 25, 50, and 100ppm, the effective concentration in another study using *Ae. aegypti* larvae of laboratory strains (17). The subject of the research was the third instar larvae offspring of the Cypermethrin-resistant *Ae. aegypti*, in intact condition, and actively moving. As many as twenty larvae were subjected to each experiment group.

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

Results

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

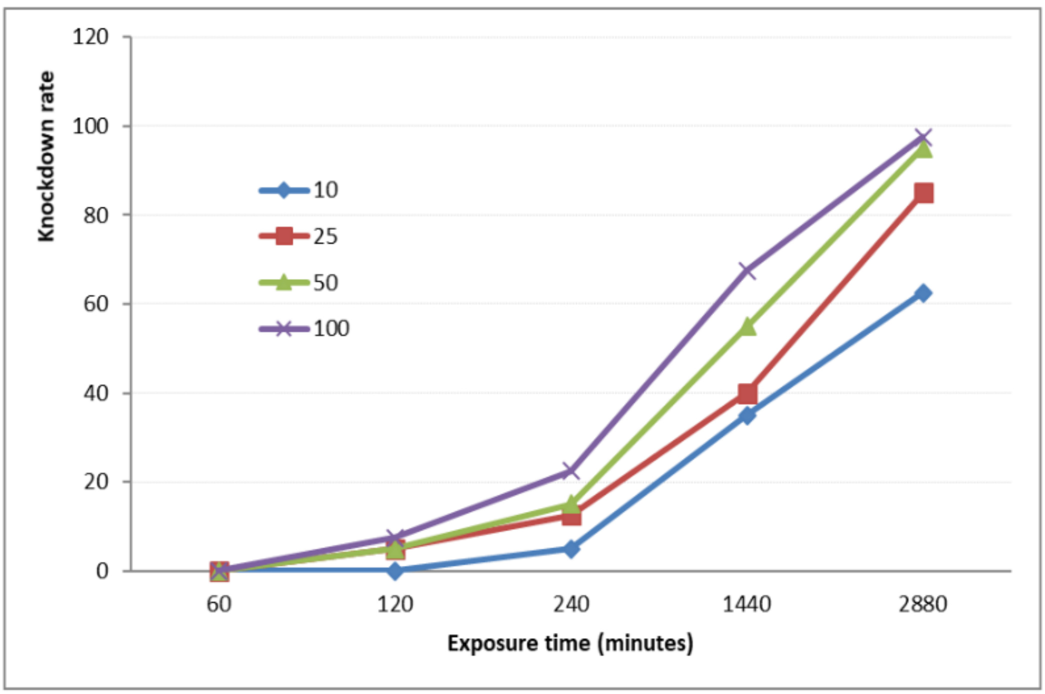

Fig. 1. The trend of larval knockdown rate in each extracts concentration based on the exposure time. The colored-line represents the concentrations of the extract

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

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

dw= distilled water; tem= temephos

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

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

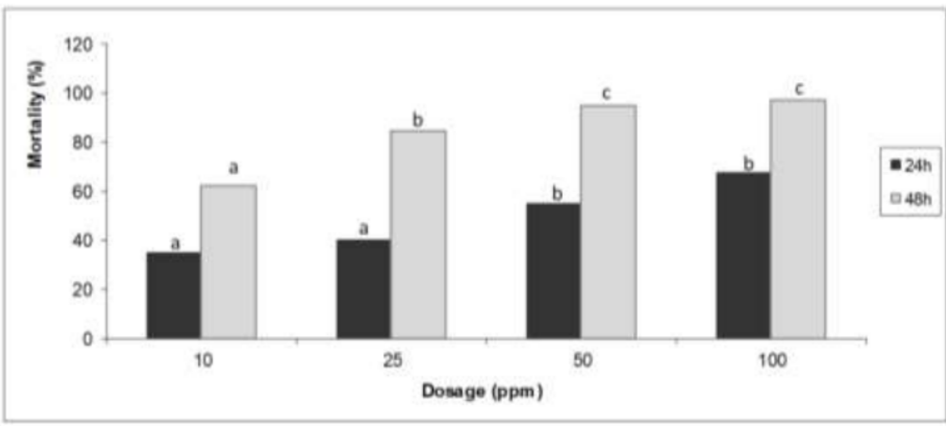

Fig. 2. Mortality rate comparison of *Aedes aegypti* larvae after 24h and 48h exposure of ethyl acetate extract of *Derris elliptica* root. The differences of larval mortality rate are indicated by the letters a, b and c

Discussion

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

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of *D. elliptica* root against the third-instar larvae of cypermethrin-resistant *Ae. aegypti* F2 offspring is 84.4% lower than the same extract potency against the susceptible (laboratory) strain (17) indicating by the LC_{50} bioassay test for 24 hours, respectively 34,945ppm and 21,063 ppm. This extract also had a higher larvicidal potential against F2 offspring larvae of Cypermethrin-resistant *Ae. aegypti* rather than the temephos-resistant offspring (18). The larvicidal activity of this extract was also better than the methanolic extract of *M. glaziovii* peel (22), *A. pinata* (23), *T. patula* (24), *H. forskaolii* (25), *O. campechianum* and *O. quixos* (26), and *A. occidentale* (27) against the third-instar larvae of *Ae. aegypti*, although lower than the specific isolate compound of *F. vulgare* (28) and *P. aduncum* (26) essential oils, and *P. foetida* ethyl acetate extract (29). These preliminary data and information have given new hope that this extract can be an alternative ingredient of larvicide to inhibit the growth of *Ae. aegypti* larvae, even to the strains that are already resistant to cypermethrin adulticide.

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

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

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

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Conclusion

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

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