ORIGINAL ARTICLE

The Effect of Red Betel Leaf's Essential Oil (Piper Crocatum Ruiz & Pav.) Against Third Instar Aedes aegypti Larvae

BUDI SETIAWAN[1]*, ICHWA ZARQYA[1], SAPTONO PUTRO[1], FURAIDA KHASANAH[1]
Poltekkes Kemenkes Yogyakarta, Indonesia,
Correspondence to Dr. Budi Setiawan, Email: budi.setiawan@poltekkesjogja.ac.id

ABSTRACT

Background :Abate SG 1gr is a larvicidal powder programmed by the government. The active ingredient is 1% temephos. However, many studies have reported cases of resistance to this insecticide. In addition to using larvicidal solutions made from chemical (synthetic) materials, many natural ingredients in our environment also have larvicidal effects, one of which is red betel leaf (Piper crocatum Ruiz & Pav.).
Aim : To determine whether the administration of red betel leaf's essential oil (Piper crocatum Ruiz & Pav.) gave death/normal/syncope effect on third instar Aedes aegypti larvae and also determining the LC50 and LC99.
Methodology and result : WHO larvicidal test's method was adopted into this experiment. Herbarium test was performed on betel leaves sample then extracted into essential oil using steam distillation method. A chemical compound of essential oil examined using GC-MS test. 25 larvae per treatment tested into four different concentrations (0.01%, 0.05%, 0.1%, 0.15%) to collect larvae mortality data after 1 hour, 3 hour, and 24 hour exposure. Data test result were processed using statistical test and probit analysis. In 24 hour exposure, concentrations 0.01%, 0.05%, 0.1% and 0.15% respectively able to eliminate sample by 29%, 84%, 100%
Conclusion : LC50 and LC99 from red betel leaf's essential oil (Piper crocatum Ruiz & Pav.) that eliminated third instar Aedes aegypti larvae in 24 hours are at 0.02425% and 0.0831%. At concentration 0.1%, red betel leaf's essential oil able to eliminate 100% of instar III larva samples in 3-hour exposure. This study concluded that red betel leaf's essential oil (Piper crocatum Ruiz & Pav.) gives death effect on the third instar Aedes aegypti larvae.
Keywords: Essential oil, Piper crocatum Ruiz & Pav., Aedes aegypti, LC50, LC99, Abate 1gr

INTRODUCTION

Indonesia nowadays has not been able to eliminate Dengue Hemorrhagic Fever (DHF). The total number of dengue cases in 2017 at Indonesia amounted to 59,047 cases with 444 of them died, and Incident Rate per 100,000 population was 22.55[1]. Dengue virus as dengue disease causes is transmitted from person to person through mosquito bites from Aedes genus, especially Aedes aegypti species which is the main vector and Aedes albopictus as the second vector. Vaccines are still being developed for prevention of dengue disease, and there are no specific medicines for healing, making DHF/Dengue Fever (DD) control dependent on eradicating Aedes aegypti mosquitoes[2]. Eradication can be done by reducing breeding sites, controlling larvae and minimizing adult mosquitoes[4]. Eradication of adult mosquitoes is still dependent on fumigation which requires high costs, making this effort has not been optimal in suppressing the number of mosquitoes in several countries[2]. Aside from eradicating adult mosquitoes directly, vector control can also be achieved by controlling larvae, one of it is by giving a toxic oil solution[4].

Abate SG 1gr is a larvicidal powder programmed by the government. The active ingredient is 1% temephos which belongs to the organophosphate group. However, many studies have reported cases of resistance to these insecticides, one of which was reported by Prasetyowati et al[5] for the scope of three municipalities in Jakarta; Chen, et al[6] reported similar results for Malaysia; Poupardin et al[7] for cases of resistance in Thailand; and Muthusamy, et al[8] for cases in India. In addition to using larvicidal solutions made from chemical (synthetic) ingredients, many natural ingredients in our environment also giving larvacidal effects. One of TOGA plants (Tanaman Obat Keluarga)[9], namely betel leaves, has been investigated for its larvicidal effects. Betel leaves are often cultivated in the home garden so that the raw material for betel leaves are easily available.

Widiyastuti examined the essential oil content of several types of betel and it was known that green betel and red betel essential oil content was 0.6%[10]. Essential oils themselves are well-known for their larvicidal effects, so red betel leaves have an opportunity as a natural ingredients to control larvae population. The essential oil of red betel leaf is also easily degradable because they are made from natural ingredients, which results in insecticides. This is in line with several previous studies. In 2017, Qodeiyaka Huda, et al. examined toxicity of red betel leaf extract (Piper crocatum) against third instar Aedes aegypti larvae. LC50 of betel leaf extraction using maceration method with ethanol 96% solvent was 322.19 ppm[10].

Considering betel leaf potential as a bioinsecticide, authors were interested in conducting research on red betel leaf which aims to determine the effect of red betel leaf essential oil (Piper crocatum Ruiz & Pav.) against third instar Aedes aegypti larvae along with finding lethal concentration 50% (LC50) and lethal concentration 99% (LC99) from red betel leaf essential oil (Piper crocatum Ruiz & Pav.) which able to kill third instar Aedes aegypti larvae in 24 hour exposure.

MATERIALS AND METHODS

This study adopted a larvicidal examination method from WHO[11]. Betel leaf samples were tested with herbarium test, then extracted into essential oils using steam distillation

method. Chemical components of essential oil examined using GC-MS method. Before doing experiment in laboratory, larvae that going to be used were first identified under microscope to ensure that larvae which were used indeed Aedes aegypti species. After that, essential oil were diluted into 0.01%, 0.05%, 0.1% and 0.15% concentration per 100ml well water, then 25 third instar Aedes aegypti larvae were placed into each dilution. Sample inclusion criteria are third instar larvae and active moving larvae. Sample exclusion criteria are larvae that already dead before treatment. The positive control solution was made using abate 1mg/100 ml well water. Negative control was made using 1 ml aquadest and 0.1 tween 80 which were dissolved in 100 ml well water. Mortality number were calculated after 24 hours exposure without being fed. Mortality number were observed regularly at 1-hour exposure, 3 hours exposure, and 24 hours exposure. The study was conducted four times.

RESULT/DISCUSSION

The essential oil extracted from red betel leaves were examined for their chemical compound using GC-MS method (table 1). Content of red betel leaf's essential oil used in this study consist of aromatic compounds and terpene compounds which were divided into monoterpenes and sesquiterpene groups. The chemical components of essential oils in the Batubara study (2011) were also known consist of terpenoids groups, which is monoterpene and sesquiterpene. This is in line with the results of essential oil's chemical components used in this study.

49.82% of the essential oils used in this study consisted of sesquiterpene group compounds. The sesquiterpene substance itself is known to be able to modify the sensile peripherals of insects that function as taste buds, resulting in reduced consumption of food. But this substance is not included in chemical compounds class that suppress appetite through the central nervous system (followed by digestion and absorption) or substances that have sub-lethal toxicity to insects.(12). Lipophilicity of terpene and phenylpropanoid compounds contained in essential oils is known to play a role in the larvicidal activity of red betel leaves essential oils. The combination of these compounds and enzyme inhibitors will increase transmembrane ability in the absorption of lipophilic drugs which can kill larvae. Elements present in essential oils affect biochemical processes, which specifically disrupt the endocrine balance of insects. Another factor is channels interference of GABA-gate chloride in insects caused by essential oil components[13].

Lipophilicity of terpene and phenylpropanoid compounds contained in essential oils is known to play a role in the larvicidal activity of red betel leaves essential oils. The combination of these compounds and enzyme inhibitors will increase transmembrane ability in the absorption of lipophilic drugs which can kill larvae. Elements present in essential oils affect biochemical processes, which specifically disrupt the endocrine balance of insects. Another factor is channels interference of GABA-gate chloride in insects caused by essential oil components[13].

However, hydrogenation of double bonds will reduce lipophilic activity by limiting the absorption of lipophilic compounds through larval cuticles[14] The presence of aliphatic alcohol and the addition of phenolic hydroxyl to lipophilic compound also reduce the activity of compounds. Pyrocatechol and resorcinol compounds show a lower activity profile. This is might be caused by lack of lipophilic group in its structure. The presence of ketones in Limonene compounds is also sufficient to reduce its activity, while the aromatization of menthol can increase compound activity by about 6-fold[13].

In a journal written by Ghosh A (2012), Rattan also discussed the mechanism of action from plant secondary metabolites on insect bodies, one of which is inhibition of the enzyme acetylcholinesterase by essential oils[15]. Inhibition of the acetylcholinesterase enzyme will cause spasms in larval muscles which, if they occur continuously, can result in the death of the larvae. This can happen becausegenerally at the endings of insects nerve also produced acetylcholine when the nerves are stimulated, which will electrical impulses stimulate the muscles to contract. This acetylcholine will normally be neutralized immediately by the acetylcholinesterase enzyme that converts it to choline, lactate and water. If not neutralized, the muscle will contract for a long time and can cause spasms[16].

Essential oils were then tested for its larvacidal effects on third instar Aedes aegypti larvae (Table 2). Water condition of concentration 0.01% did not change too much, but there was slight change in colour at concentration 0.15%. The effect of the treatment using essential oils also provides a refreshing aromatherapy so that it can also be used for relaxation.The difference in mortality number from four concentrations of essential oils might be caused due to differences in the amount of active ingredient in each concentration. Essential oils with higher concentrations contain more active ingredients than essential oils with low concentrations.

Table 2. The contents of the essential oils of red betel leaves

Compound	Category	%age
β-Phellandrene	Monoterpena	5,81
1,8 Cineole		3,04
α-Thujene		2,46
γ-Terpinene		2,45
trans-Sabinene hydrate		0,60
Menoterpena		**14,36**
α-Selinene	Sesquiterpena	9,97
β-Selinene		8,51
α-Humulene		8,05
Germacrene D		6,91
Germacrene B		1,05
δ-Cadinene		4,28
α-Cubebene		0,60
trans-Charyophellyne		10,45
Sesquiterpena		**49,82**
Naphthalene	Aromatik	9,61
Phenol		5,23
Aromatik		**14,84**
Manganese	Mineral	1,62
Chlavicyl acetate	Phenylpropene	8,91
Bicyclo-nonane	Lainnya	7,66
1H-3a, 7-Methanoazulene		2,94

At 3 hour exposure, concentration 0.1% has been able to kill 100% of larvae, while positive control was only able to kill 67% of larvae. This shows that the performance of the red betel leaf essential oil in killing larvae is faster

than positive control (Terpehos), although at the end of the 24-hour exposure, both of them kill 100% of larvae.

The results of the probit analysis in 24-hour exposure showed that LC50 (Lethal Concentrate 50%) was at concentration 0.02425% and LC99 (Lethal Concentrate 99%) was at concentration 0.0831%. This result is higher than previous research conducted by Qodeiyaka Huda, et al (2017)(10) who obtained LC50 of betel leaf extraction using maceration method with ethanol 96% solvent was at 322.19 ppm or 0.03%. This might be caused by the different extraction methods used in each study.

Based on experiment results, it is visible that red betel leaf essential oil can be used as a larvacidal alternative product against third instar Aedes aegypti larvae, and statistically, there are differences in mortality number at various concentrations used. The higher the concentration, the faster and the higher mortal rate of larvae caused by the essential oil of red betel leaf.

Table 2. Number of Death of Instar III Larva Aedes aegypti After 1.3 and 24 Hours Treatment with Essential Oil of Red Betel Leaves in Various Concentrations

Repetition	Group					
	Control (+)	Concentration of Red Betel Leaves Essential Oil				Control (-)
		0,01%	0,05%	0,1%	0,15%	
1 Hour Exposure						
1	15	0	6	18	20	0
2	15	0	7	16	20	0
3	18	0	9	16	23	0
4	18	0	8	20	22	0
Average	16,5	0	7,5	17,5	21,25	0
Percentage(%)	66	0	30	70	84,88	0
3 Hour Exposure						
1	16	4	20	25	25	0
2	15	5	18	25	25	0
3	18	5	19	25	25	0
4	18	6	20	25	25	0
Average	16,75	5	19,25	25	25	0
Percentage(%)	67	20	77	100	100	0
24 Hour Exposure						
1	25	7	20	25	25	0
2	25	8	21	25	25	0
3	25	6	22	25	25	0
4	25	8	21	25	25	0
Average	25	7,25	21	25	25	0
Percentage(%)	100	29	84	100	100	0

CONCLUSION

At concentration of 0.1%, essential oil of red betel leaves able to kill 100% larvae in 3-hour exposure. Lethal concentration 50% (LC50) and Lethal concentration 99% (LC99) of essential oil red betel leaves (Piper crocatum Ruiz & Pav.) which able to kill third instar larvae of Aedes aegypti in 24-hour exposure are at 0.02425% and 0.0831%

REFERENCES

1. Kemenkes RI. Data dan Informasi Profil Kesehatan Indonesia 2017. Accessed July 1,2018.
2. WHO-DEPKES. Pencegahan dan Penanggulangan Penyakit Demam Dengue dan Demam Berdarah Dengue. Accessed July 1, 2018.
3. Sembel Dantje T. Entomologi Kedokteran. Yogyakarta: C.V Andi Offset; 2009.
4. Brown Harold W. Dasar Parasitologi Klinis. Jakarta: PT. Gramedia Pustaka; 1983.
5. Prasetyowati H, Hendri J, Wahono T W. Status Resistensi Aedes aegypti (Linn.) terhadap Organofosfat di Tiga Kotamadya DKI Jakarta. Jurnal BALABA. 2016;12(1):23-30.
6. Chen CD, Nazni WA, Lee HL, Norma-Rashid Y, Lardizabal ML, Sofian-Azirun M. Temephos resistance in field Aedes (Stegomyia) albopictus (Skuse) from Selangor, Malaysia. Trop Biomed. 2013 Jun;30(2):220-230.
7. Poupardin R, Srisukontarat W, Yunta C, Ranson H. Identification of carboxylesterase genes implicated in temephos resistance in the dengue vector Aedes aegypti. PLoS Neglected Tropical Disease. 2014 Mar;8(3):e2743.
8. Muthusamy R, Shivakumar MS. Susceptibility status of Aedes aegypti (L.) (Diptera: Culicidae) to temephos from three districts of Tamil Nadu, India. J Vector Borne Dis. 2015 Jun;52(2):159-65.
9. Departemen Pertanian. Keputusan Menteri Pertanian RI No. 511/Kpts/PD.310/9/2006 tentang Hortikultura. Accessed July 1, 2018.
10. Qodeiyaka, Huda. Toksisitas Ekstrak Daun Sirih Merah (Piper crocatum) Untuk Mematikan Larva Instar III Sebagai Vektor Dengue Nyamuk Aedes aegypti. Skripsi. Jurusan Farmasi FMIPA Universitas Pakuan. 2017.
11. WHO. Guideline for Laboratory and Field Testing of Mosquito Larvicides. WHO/CDS/WHOPES/GCDPP/2005.13
12. Adeyemi MM Hassan. The Potential of The Secondary Metabolites in Plant Material as Deterents against Insect Pest : A Review. Afr.J.Pure Appl. Chem. 2010 Des;4(11):243-246.
13. Santos HS, Paulo NB, Telma LGL, Gilvandete MPS. Chemical composition and larvicidal activity against Aedes aegypti L. (Diptera: Culicidae) of essential oils from leaves, stalks and roots of the Croton nepetaefolius Baill (Euphorbiaceae). International Journal of Mosquito Research. 2017;4(5):19-22.
14. Santos SRL, Silva VB, et al. Larvicidal activity of terpenes against Aedes aegypti. 4th Brazilian Symposium on Medical Chemistry. 2008.
15. Ghosh A, Chowdhury N, Chandra G. Plant extract as potential mosquitos larvicides. Indian J Med Res. 2012;135(5):581-598.