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Potential of turmeric rhizome essential oils against *Aedes aegypti* larvae

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ABSTRACT

BACKGROUND

Dengue hemorrhagic fever (DHF) has long been a serious health problem in Indonesia, including Kalimantan (Borneo), as is evident from the increased case fatality rate in Banjarbaru city. Synthetic chemical insecticides have frequently been used to eradicate mosquitoes, but are toxic to the body and resistance of adult and larvae mosquito *Aedes aegypti* has been reported. The present study aims to assess the effect of essential oils of turmeric rhizomes (*Curcuma domestica* Val) against *Aedes aegypti* larvae

METHODS

This was an experimental study of post test one group design, performed in two phases, using *Aedes aegypti* larvae as test organisms. In the first phase, laboratory-reared larvae were used for calculation of the LC_{50} and $LC_{90,}$ while in the second phase the test organisms were larvae taken from 75 buildings that had been designated based on a preliminary survey in four sub-districts in Banjarbaru city with a high incidence of dengue cases. Probit analysis of was used to calculate LC_{50} and LC_{90}, and the Kruskal-Wallis test to determine the larvicidal potency of turmeric rhizome essential oils.

RESULTS

This study demonstrates that turmeric rhizome essential oils effectively killed laboratory-reared *Aedes aegypti* larvae at an LC_{50} of 9.239 ppm and an LC_{90} of 13.565 ppm. The effectiveness of the essential oils of turmeric rhizomes (*Curcuma domestica* Val.) for killing *Aedes aegypti* larvae in residential areas was 68%.

CONCLUSION

Turmeric (*Curcuma domestica* Val.) rhizome essential oils can kill *Aedes aegypti* larvae**,** are environment friendly and can be used for the control of mosquitoes.

Keywords: turmeric rhizome essential oils, larvicidal, *Aedes aegypti* larvae

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Potensi larvisida minyak atsiri rimpang kunyit terhadap larva Aedes aegypti

ABSTRAK

LATAR BELAKANG

Demam berdarah dengue (DBD) menjadi salah satu masalah kesehatan di Indonesia, termasuk Kalimantan, seperti terlihat dari case fatality rate *di Kota Banjarbaru yang semakin meningkat. Insektisida kimia sintetis sering digunakan untuk pemberantasan nyamuk, namun bahan kimia ini bersifat toksik bagi tubuh. Beberapa laporan menyatakan terjadinya resistensi nyamuk dewasa dan larva Aedes aegypti terhadap insektisida tersebut. Penelitian ini bertujuan untuk menilai pengaruh minyak atsiri rimpang kunyit (Curcuma domestica Val.) terhadap larva Aedes aegypti.*

METODE

Rancangan penelitian yang digunakan adalah eksperimental dengan pendekatan post test one group design. *Obyek penelitian adalah larva Aedes aegypti. Tahap pertama larva yang digunakan dari hasil kolonisasi di laboratorium untuk menghitung LC_{50} dan LC_{90} dan tahap kedua larva diambil dari 75 rumah yang sudah ditetapkan berdasarkan survey pendahuluan di empat kecamatan di wilayah Kota Banjarbaru yang mengalami kasus DBD tinggi. Analisis data yang digunakan adalah uji probit untuk menghitung LC_{50} dan LC_{90}, dan uji Kruskal-Wallis untuk menguji daya larvisida minyak atsiri rimpang kunyit.*

HASIL

Studi ini menunjukkan bahwa minyak atsiri efektif membunuh larva Aedes aegypti yang ditangkar di laboratorium, dengan LC_{50} sebesar 9,239 ppm dan LC_{90} sebesar 13,565 ppm. Efektivitas minyak atsiri rimpang kunyit (Curcuma domestica Val.) untuk membunuh larva Aedes aegypti di lingkungan perumahan adalah 68%.

KESIMPULAN

Minyak atsiri rimpang kuyit (Curcuma domestica Val.) mampu membunuh larva Aedes aegypti, ramah terhadap lingkungan sehingga dapat digunakan untuk pengendalian nyamuk.

***Kata kunci** : Minyak atsiri rimpang kunyit, larvisida, larva Aedes aegypti*

INTRODUCTION

Dengue hemorrhagic fever (DHF) is a disease caused by the dengue virus and transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. In Indonesia DHF has been a public health problem for the last 41 years. The number of provinces and districts endemic for DHF has increased from 2 provinces dan 2 cities in 1968 to 32 provinces (97%) and 382 districts (77%) in 2009. In 2007, 2008 and 2009 the number of DHF cases occurring was 158 115, 137.468 and 158.912, respectively.[1] The incidence rates in South Kalimantan was 35.59/100,000 inhabitants in 2007, 14.44/100.000 inhabitants in 2008, and 11.26/100,000 in 2009 (January-September). In Banjarbaru city the incidence rate in the years 2007–2009 was 45.10/100,000, 34.30/100,000, and 52.09/100,000, respectively, while in 2010 (from January until Sptember) the incidence rate was 113.9/100,000 inhabitants. The case fatality rate (CFR) of DHF in Banjarbaru city was as follows: 1.9% (2006), 1.8% (2007), 1.9% (2008), 5.11% (2009), and 2% (until September 2010).[2-3]

At present there are no drugs and vaccines available for dengue virus eradication in connection with DHF prevention. The disease may be most appropriately managed by eradicating the mosquito vectors. Eradication of *Aedes aegypti* may be conducted by killing mosquito larvae by means of larvicides. The most widely used larvicide for control of *Aedes aegypti* larvae is Temefos 1% (Abate 1SG).[4-6]

Synthetic insecticides are used by the community because they are practical in use and rapid in action. However, the use of synthetic insecticides has not led to a reduction in DHF rates. On the contrary, there are reports from many countries about the occurrence of insecticide resistance, environmental pollution, and contamination of humans and animals. Most of the available synthetic insecticides kill only adult mosquitoes, and only a few kill mosquito larvae.[7-9] Previous studies have demonstrated that several plants, such as turmeric (*Curcuma domestica* Val.), are a potential alternative source of bioactive phytochemicals for killing *Aedes aegypti* larvae.[10-12] These plant-derived insecticides may be expected to succeed in replacing conventional insecticides, as they are believed to be target-selective or target-specific. In contrast to synthetic insecticides, these phytochemicals are degradable to nontoxic compounds, thus minimizing the harmful effects on humans and animals, and contributing to a higher degree of environmental safety by minimizing the accumulation of harmful residues in the environment For this reason, they are also potentially suitable for use in the continuation of integrated mosquito control programs.[13-15]

Based on the abovementioned considerations, the study and development of alternative larvicides that are environment-friendly is clearly indicated to decrease the use of synthetic insecticides. These plant-derived larvicides are expected to result in a reduction of the number of DHF cases in South Kalimantan, especially in Banjarbaru City. The alternative candidate larvicides used in the present study are derived from indigenous Indonesian plants, such as turmeric, which is readily available, inexpensive, and highly effective. In 2009, Panghiyangani et al.[11] concluded that 0.4% ethanolic extract of turmeric rhizome *(Curcuma domestica* Val.) was an effective larvicide against *Aedes aegypti* in residential areas of Banjarbaru. Other studies demonstrated that the essential oils contained in a number of medicinal plant parts, such as the leaves of Pandanus spp., *sirih* (*Piper betle* Linn), lemon grass (*Andropogen nardus*), *Eucalyptus cinerea*, and the rhizomes of white turmeric (*Curcuma zedoaria*), were capable of killing *Aedes aegypti* larvae.[8,16,17] The aim of the present study was to evaluate the effects of turmeric rhizome essential oils (*Curcuma domestica* Val) on *Aedes aegypti* larvae.

METHODS

Design of study

The study was of experimental design using a post test control group approach and was conducted from May to November 2010.

Extraction of turmeric rhizome essential oils

Approximately 10 kg of turmeric *(Curcuma domestica* Val.) rhizomes was cut into small pieces and placed in the steam distillation apparatus containing 10 liter water, and connected to a condensor. The steam distillation apparatus was then heated, with steps being taken to avoid overheating and to maintain the flow of steam to the condensor. The condensor was kept cool by external packing with ice, to ensure condensation of all essential oils in the water phase.

The oil-and-water mixture in the distillate was subsequently separated by means of a separatory funnel. For a complete separation, sodium chloride was added to the distillate. The water phase was collected in an erlenmeyer flask for further separation of remaining traces of oils, and after addition of sodium chloride the water was decanted, then separated in the separatory funnel.[18]

Larvicide bioassay

The study was performed in 2 stages, with the first stage performed in the laboratory using laboratory-reared larvae for determination of LC_{50} and LC_{90}, to serve as the basis for calculating the treatment dosage be used in the field. For a mortality of *Aedes aegypti* larvae between 0% and 20%, the number of deaths in the intervention groups was corrected using Abbot's formula:[19,20]

$$AI = \frac{\%\ \text{intervention mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} X\ 100$$

AI: mean corrected persentage of deaths of test larvae

The control groups were 2 in number, i.e. positive controls (abate 1%) and negative controls sodium carboxymethylcellulose [CMC-Na] 0.5%. There were seven intervention groups, each consisting of 25 larvae, to whom the essential oils were administered at concentrations of 3.45, 6.9, 13.79, 27.5, 55, 110, and 220 ppm, respectively. The treatment was replicated three times and the larvae were observed for 24 hours. The second phase of the larvicidal potency test used *Aedes aegypti* larvae collected from 75 buildings selected in preliminary surveys, and consisting of 3 groups of 25 larvae, i.e. positive controls, negatiuve controls, and the intervention group receiving turmeric essential oils at a concentration of 13.565 ppm, based on the LC_{90} obtained in the first stage of this study, on the expectation of 100% larval mortality. These larvae were also observed for 24 hours.

The effectiveness of turmeric rhizome *(Curcuma domestica* Val.) essential oils in comparison to the positive control groups (Temefos 1%) was calculated with the following formula:

$$\text{Effectiveness} = \frac{\text{Mean mortality of intervention larva}}{\text{Mean mortality of control larvae}} X\ 100$$

Statistical analysis

Probit analysis was performed using SPSS 17 to calculate LC_{50} and LC_{90} values and the Kruskal-Wallis test for determining larvicidal potency of turmeric rhizome essential oils against *Aedes aegypti* larvae, at a significance level of 5%.

RESULTS

Probit analysis found an LC_{50} value of 9.239 ppm and an LC_{90} of 13.565 ppm, as shown in Table 1. Larval mortality in the group given turmeric rhizome essential oils was 65.22%, after Abbot's correction.

The results of the larvicidal assay of turmeric rhizome essential oils against *Aedes aegypti* are shown in Table 2. The results of the Kruskal-Wallis test showed a significant difference in larval mortality between the three treatment groups (p=0.021). Larval mortality in the group given essential oils was 22% (17/75), which was significantly lower than the larval mortality of 33% (25/75) in the group given Abate 1%. The effectiveness of turmeric *(Curcuma domestica* Val.) rhizome essential oils as larvicide, in comparison with the positive control group (Temefos 1%) was 0.68.

DISCUSSION

The first phase of this study found LC_{50} and LC_{90} values of 9.239 ppm and 13.565 ppm, respectively, for turmeric *(Curcuma domestica* Val.) rhizome essential oils as larvicide (Table 1). The LC_{50} in our study was lower than the 54.5 ppm for the LC_{50} of white turmeric essential oils.[21] A Thai study also found the higher LC_{50} value of 36.30 ppm for *Curcuma aromatica* essential oils.[15] Thus our differing study results show that turmeric essential oils have a lower larvicidal effect than that of *Curcuma aromatica*.

The results of Kruskall-Wallis test showed that turmeric essential oils had the capacity to kill *Aedes aegypti* larvae, although their potency

Table 1. Distribution of larvacidal activity of turmeric rhizome essential oils against *Aedes aegypti*

	Probability	95% Confidence Limits		
		Estimate	Lower Bound	Upper Bound
PROBIT(a)	.010	1.384	-1.010	2.943
	.020	2.305	.170	3.719
	.030	2.889	.915	4.215
	.040	3.328	1.472	4.592
	.050	3.685	1.922	4.901
	.060	3.989	2.304	5.165
	.070	4.256	2.637	5.399
	.080	4.495	2.934	5.610
	.090	4.712	3.202	5.803
	.100	4.912	3.448	5.981
	.150	5.739	4.453	6.736
	.200	6.397	5.230	7.356
	.250	6.961	5.880	7.905
	.300	7.468	6.447	8.415
	.350	7.938	6.957	8.903
	.400	8.383	7.428	9.379
	.450	8.814	7.870	9.853
	.500	**9.239**	8.293	10.331
	.550	9.663	8.706	10.820
	.600	10.094	9.116	11.326
	.650	10.539	9.531	11.859
	.700	11.009	9.959	12.428
	.750	11.516	10.413	13.051
	.800	12.080	10.910	13.753
	.850	12.738	11.481	14.580
	.900	**13.565**	12.189	15.631
	.910	13.765	12.358	15.886
	.920	13.982	12.542	16.164
	.930	14.221	12.744	16.470
	.940	14.488	12.968	16.812
	.950	14.792	13.223	17.204
	.960	15.149	13.522	17.664
	.970	15.588	13.888	18.232
	.980	16.172	14.373	18.988
	.990	17.093	15.133	20.183

Table 2. Mortality percentage of *Aedes aegypti* in the treatment groups

	Treatment			p
	Negative Controls (n=75)	Turmeric rhizome essential oils (n=75)	Positive Controls (n=75)	
% mortality	2	17	25	0.021

was less than that of Temefos 1% (Abate), as not all larvae were killed. This may be due to the use of a concentration killing 90% of the laboratory-reared larvae (LC_{90}) within 24 hours, whereas the test was done in residential areas, the natural habitat of *Aedes aegypti*.

There was a difference in susceptibility between laboratory-reared larvae and wild-type larvae from residential areas with regard to application of turmeric rhizome essential oils. The effectiveness of turmeric (*Curcuma domestica* Val.) rhizome essential oils in killing *Aedes aegypti* larvae from residential areas was two-thirds that of the positive controls. On the basis of the laboratory tests, the results of the effectiveness test should have been more than 68%, because the concentration used was the LC_{90} (13.565 ppm). There is thus a difference of 22% between laboratory and field test results. Since essential oils are volatile compounds, and the field tests were performed in the afternoon hours without practically any control over the temperature of the respective buildings, it may be surmised that a substantial amount of the essential oils was lost through evaporation when pouring the oils into the test containers. This is in contrast with the laboratory tests, which were performed at controlled lower temperatures, thus minimizing loss of oils through evaporation.

The rhizomes of turmeric (*Curcuma domestica* Val.) contain the following active substances: curcumins, sesquiterpenes, turmerones, volatile oils (essential oils), and zingiberens, turmerols, phellandrenes, camphors, curcumons, and various resins with antibacterial properties.[22] According to the studies of Heyne,[23] derivatives of oxygenated hydrocarbons (phenols) have strong antibacterial properties. Phenolic compounds mainly act by denaturation of cellular proteins and damage to cell membranes. The phenolic content of a substance may result in lysis of larval cells, due to increased permeability of cell membranes, leading to leakage of essential metabolites, while in the cells the phenols disrupt cellular activity. Phenolic compounds act as dessicants, and are contact poisons that kill by inducing a continuous leakage of fluids, causing the larvae that come in contact with these poisons to die from dehydration. A contact poison is a larvicide that enters the larvae through the integument and natural orifices (siphons). The larvae die on direct contact with the larvicide. Most contact poisons also act as stomach poisons.[24]

One limitation in this study was that the stability of turmeric rhizome essential oil preparation could not be maintained over time, due to the volatility of the oils.

CONCLUSION

Turmeric rhizome essential oils (*Curcuma domestica* Val.) can kill *Aedes aegypti* larvae from residential areas in several subdistricts in Banjarbaru city. We recommend that the larvicidal effect of turmeric rhizome essential oils on *Aedes aegypti* larvae be utilized in DHF vector control.

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