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Larvicidal activity of different solvent extracts of *Leucaena leucocephala* againts *Aedes aegypti*

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Abstract
Aedes aegypti is a vectors transmitting any serious disease that cause morbidity and mortality in human. Insecticides not only failed to control of insect development but also led to development of mosquitoes resistance. Plant-derived products is being one way to resolve these problem. An insecticide does not need to cause high mortality on taget organisms but should be eco-friendly in nature. This study was conducted to test the efficacy of various extract of *Leuchaena leucocephala* against *Ae. aegypti*. The ethanol, methanol, and N hexane leaf of *L. leucocephala* were studied in the range of 0,125 to 1,5% in the laboratory bioassays against early 4th instar larvae of *Ae. aegypti*. The mortality data were analyze with probit to determine the lethal concentration (LC_{50} and LC_{90}) to kill 50 and 90 percent of treated larvae. The highest larval mortality was found in N hexane leaf extracts with LC_{50} values was 0,305%; leaf ethanol and methanol extracts with LC_{50} values of 1,074 and 1,025%. N hexane extract outstand as highly active larvicide, achieving 91% larval mortality in 24 mortality hours according to the WHO protocol. These results suggest that the effective plant crude extracts of N hexane have the potential to be used as an ideal ecofriendly approach for the control of the *Ae. aegypti*.

Keywords: *Leucaena leucocephala*, larvicidal, *Aedes aegypti*

1. Introduction
Aedes aegypti (*Ae. aegypti*) play an important role in the transmission of dengue fever, chikungunya, yellow fever, filariasis, zika, Japanese encephalitis, and several diseases which are today the greatest health problems in the world. *Ae. aegypti* is the vectors for the pathogens of various diseases especially in tropical countries. Those patogens can alife and multiply in the mosquito's body. The development of *Ae. aegypti* depend on traveling, climate, temperature, global warming, and tainted fresh water container increasing the breeding of vectors [1-3].

To prevent proliferation of mosquito borne diseases and to improve quality of environmental and public health, mosquito control is essential. One of the approaches for controlling mosquitoes borne diseases is interruption of diseases transmission by killing, preventing mosquito bite using repellents or using larvicidal agent at the breeding center of the vector. The control of *Ae. aegypti* larvae worldwide is by using organophosphate insecticide [4]. Repeated use of chemical insecticides for mosquito control in long term can lead to resistance, ecological imbalance, harm to human and animal [5-8].

The mosquito control at the larval stage with phytochemical that occur in the leaves, fruit, roots and oils of plants is one of the techniques which is eco-friendly. Besides that the use of botanicals are biodegradable and nontoxic [5]. Phytochemical are now recognized as potent alternative insecticides for mosquito replacing chemical insecticides.

Leucaena leucocephala (*L. leucocephala*) belonging to *Mimosaceae* family is widely distributed in tropical regions. It is used in South East Asia for its medicinal properties [4]. Based on previous study *L. leucocephala* leaves can also be used as an antioxidant, antimicrobial, energy sources, and the sources of human food or animal [9, 10]. The antimicrobial properties of the *L. leucocephala* have been demonstrated against gram's positive and gram's negative bacteria [11]. Leave extracts showed antioxidant, antidiabetic and anticholinesterase activity, mainly due to the reduction of oxidative stress in impaired fasting blood glucose patients [12]. Cytotoxic activity of flavonoid glycosides isolated from *L. leucocephala* has been demonstrated [13]. Other biological properties such adipogenesis, lipolysis and glucose uptake activities of fruit aqueous extract of *L. leucocephala* have been

reported [14]. A recent study demonstrated the nutritive value and potential uses of *L. Leucocephala* as a biofuel [15, 16].

In recent years, much effect has been focused on the exploration of bioactive, chemical compounds from indigenous plants for mosquito control. Despite the important biological properties described, these plants have never been investigated as potential source of larvicidal molecules effective against mosquitoes. In this study, leaf extract of the *L. leucocephala* were tested against the larvae of *Ae. aegypti*. This study was aimed to look at the potential of ethanolic, methanolic and N-Hexane extract of *L. leucocephala* against early 4th instar of *Ae. aegypti* as a larvicide. The results of the present study would be useful in promoting research aiming toward the development of new agents for mosquito control based on bioactive chemical compounds from indigenous plant sources.

2. Materials and Methods

2.1 Mosquitoes rearing

Aedes aegypti larvae were obtained from Insectary of Parasitology department Universitas Islam Indonesia. The larva were maintained at 24±5°C and 60±3% relative humidity under 12:12 light and dark cycles. Larvae were fed a dry chicken liver.

2.2. Plants materials

The leaf of *L. leucocephala* were collected from rural areas located in north part of Yogyakarta Province, Indonesia and the taxonomic identification and authenticated by the Pharmacognosy Department, Faculty of Biological and Chemical Science, Universitas Islam Indonesia.

2.3 Preparation of plants extracts

The leaf were dried at the environmental temperatures. The dried leaf (550 gr) for each solvent were powdered using blender and extracted with ethanol, methanol and N hexane in a soxhlet apparatus for 10h. All the extract were concentrated using rotary flash evaporator under pressure 21-25 mmHg at 45ºC. The residue obtained was stored at 4ºC in airtight bottle until further use. Variations test concentration in the main test were obtained from a preliminary test to get a percentage of 10-90% larvae mortality.

2.4 Larvicidal test

A total of three trial were carried out with four replicate per trials against mosquito. Stock solution was prepared by dissolving of crude extract in emulsifier and volume raised to 100 ml with distilled water. From the stock solution different dissolution of 0,25; 0,5; 0,75; 1; 1,5% for ethanolic extract; 0,25; 0,5; 0,75; 1; 1,125% for methanolic extract and 0,125; 0,2; 0,25; 0,3; 0,5% for N-Hexane extract respectively according to standard WHO procedures. Twenty five early fourth instar larvae were released and mortality was scored after 24h. The beakers were kept in a temperature control room at 24ºC±5ºC and the larvae exposed to emulsifier of served as control for each extract. Larvae said to be died if it does not move in with a stick and light stimulation. The mortality of larvae was calculated by counting the number of dead larvae divided larvae test in percent.

2.5 Phytochemical screening

The phytochemical test were carried out from ethanol, methanol and N-Hexane extract to analyze the phytochemical constituents such as alkaloid, saponin, tannin, flavonoids. By this analysis, the presence of several phytochemical listed in Table 2.

2.6 Statistical analysis

The percentage mortality observed and corrected using Abbott's formula during the observation of larvicidal potential of plant extract. The average larval mortality data were subjected to probit analysis to find out LC_{50} and LC_{90} values. Other statistic at 95 percent of upper confident limit and lower confident limit. Analysis of variance (ANOVA) was performed to find the significant differences between the solvents and on larval mortality. Mortality data were tested for normality (Shapiro-Wilk) to analysis.

3. Results and Discussion

Plants as potential larvicides were consider to preferred alternative mosquito at the community level. Larvicidal agents is a major component for control vector borne diseases. A large number of plants extracts have been reported to have larvicidal activities against mosquito vector [17-21].

Three different solvent (ethanol, methanol and N hexane) of *L. leucocephala* were examined for their larvicidal activity against *Ae. aegypti*. The result showed that N hexane crude extract caused higher mortality followed by ethanol and methanol extract of *L. leucocephala*. The LC_{50} and LC_{90} values obtained from probit analysis for mortality values after 24h. The results obtained are presented in Table 1.

Table 1: Mortality percentage, LC_{50} and LC_{90} to different concentration of various plant extract of *L. leucocephala* againts *Ae. Aegypti*

Solvents	Concentration (%)	24 hr % mortality	*P- value*	LC_{50} (%) (LCL-UCL)	LC_{90} (%) (LCL-UCL)
Ethanol	0,25	12	0.000	1,074 0,645-1, 345	5,601 3,400-28, 456
	0,5	17			
	0,75	33			
	1	49			
	1,5	72			
Methanol	0,25	20		1,025 0,485-0, 620	1,619 1.558-2.740
	0,5	44			
	0,75	56			
	1	72			
	1,25	76			
N-Hexane	0,125	14		0,305 0, 286-0, 329	0,579 0, 509-0, 692
	0,2	19			
	0,25	29			
	0,3	39			
	0,5	91			

LC_{50}-Lethal concentration that kills 50% of the exposed larvae; LC_{90}-Lethal concentration that kills 90% of the exposed larvae; LCL-lower confidence limit; UCL-upper confidence limit

Control-nil mortality

The N hexane extract of *L. leucocephala* at concentration 0.5% showed significantly maximum larvicidal activity with LC_{50} and LC_{90} were 0.305% and 0.579%. This was followed by methanol extract with LC_{50} and LC_{90} were 1.025% and 1.619%. The crude extracts from ethanol showed lower larval mortality with LC_{50} and LC_{90} were 1.074% and 5.601%. The N hexane extract was responsible for the strong mortality activity and statistically significant than ethanol and methanol extracts (p=0.000) as reported [22, 23]. All the other extracts (ethanol and methanol) were also effective but at a slightly higher concentration. As shown in Table 1, ANOVA analysis indicate that there were significant differences in larvicidal effects between solvent (P<0.05).

The result indicate that the N hexane extract of *L. leucocephala* leaf showed the best larvicidal efficacy towards *Ae. aegypti* larvae and no mortality was recorded in the control treatment. Our finding also showed that the rise in concentration of the crude extracts caused an increase in percentage mean mortality of larvae (Table 1). The variation in the larvicidal potentiality of crude extract was vary according to plants part, concentration, species, and solvent used [23, 24]. Torres *et al*. 2014 reported the efficacy of N hexane extract of *Persea Americana* Mill. exhibited the highest toxicity with LC_{50} and LC_{50} value of 9.82 mg/L and 22.19 mg/L, while the ethanol extract exhibited LC_{50} of 16.48 mg/L and LC_{50} 45.7 mg/L after 24h of exposure period [25].

Phytochemical derived from plants extracts act as general toxicants against larval stages of mosquitoes. The preliminary phytochemical screening of leaf extract revealed the presence of saponin, tannin, alkaloid and flavonoid were effective against early 4th instar larvae after 24h of exposure (Table 2) [25]. These compounds may jointly or independently contribute to produce larvicidal activity.

Table 2: Phytochemical screening of plant extracts of *L. Leucocephala*

Type of extract	Phytochemical constituent	Qualitative analysis
Ethanol	Alkaloid	+
	Saponin	+
	Tannin	+
	Flavonoids	+
Methanol	Alkaloid	+
	Saponin	+
	Tannin	+
	Flavonoids	+
N-Hexane	Alkaloid	+
	Saponin	+
	Tannin	+
	Flavonoids	+

Many researchers successfully started that phytochemicals plays a major role to decrease mosquito population trough larviciding [17-21]. Bioactivity of plant-based insecticides against mosquito larvae vary according to solvent used in extraction [19, 21, 26, 27]. Previous studies reported the effect of flavonoid on mosquito larvae [17, 18, 20, 28]. A saponin belonging to group flabelliferin from palmyrah flour was found to be lethal to dengue mosquitoes [29]. Alkaloid are generally knew to be important sources of potent insecticide for mosquito control [30]. Tannin are also credited with insecticide activities [31].

In the present study the different solvent extracts of *L. leucocephala* leaf showed very high, moderate and low larvicidal effects. However, highest mortality was observed in N Hexane when compare to other extract. These result are in accordance with other studies [25, 32]. The larvicidal property of the leaf extract may be due to the presence of alkaloid, flavonoid and tannins compounds [25, 30, 31, 32].

The bioactivity of plant-based larvicide against mosquito larvae vary significantly according to solvent used in extraction [33]. In the present study, N hexane, ethanol and methanol were used since they have different polarities which lays in line with this study [34, 35]. Polar solvents produced moderate larvicidal bioassay result corresponding with the present findings [36].

4. Conclusion

The efficacy of different solvents of *L. leucocephala* was tested against *Ae. aegypti* under the field conditions. Among all, the N hexane extract was found most superior as larvicide followed by methanol and ethanol extract. This study also confirms the presence of secondary metabolites likes alkaloid, saponin, tannin in the solvent extracts which could be used as alternative for control of mosquito borne-diseases integrated with vector control programs. Further studies required to know of action of active ingredients. This findings suggest that this bioactive compound can be used as prototypes for larvicidal agents.

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