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Larvicide of *Aedes aegypti* (Diptera: Culicidae) from *Ipomoea pes-caprae* (Solanales: Convolvulaceae)

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Abstract. This research aimed to evaluate larvicidal candidate of the extracts of whole parts (roots, stems, leaves, flowers, and seeds) of *Ipomoea pes-caprae* (L.) R. Br. on *Aedes aegypti* (Linnaeus, 1762) larvae. The criteria applied to select larvicidal candidate were (1) the concentration of the extract solution must be ≤ 50 ppm, and (2) the larval mortality due to administration of the extract should be reached ≥ 75%. The *I. pes-caprae* parts were extracted with methanol and water solvents. Refer to the criteria, the methanol extract of the *I. pes-caprae* leaf was selected as the larvicidal candidate of the *A. aegypti* larvae. The 3rd instar of *A. aegypti* larvae was tested with five kinds of concentration of an aqueous solution of *I. pes-caprae* leaf extracts by completely random design with four replications. The methanol extract of *I. pes-caprae* leaf showed a very strong larvicide (LC_{50} was 12.60 ppm) of *A. aegypti* larvae.
Key Words: larvae, *Aedes aegypti*, *Ipomoea pes-caprae*, instar, larvicidal candidate, methanol extract.

Introduction. Dengue Hemorrhagic Fever (DHF) is a disease spread by the *Aedes aegypti* (Linnaeus, 1762) mosquito with a rapid rate of transmission and occurs in tropical regions, subtropical, and temperate in the whole world. DHF is one health problem in the world which the number of sufferers have been gradually increasing in quantity (Rao et al 2011). One of the efforts to fight against the disease is to control dengue vector. The *A. aegypti* is the major vector in the spread of dengue disease (Gubler 1997). Eradication of *A. aegypti* is usually done by way of termination of the mosquito life cycle by killing the mosquito larvae. The eradication of *A. aegypti* mosquito larvae is generally done using abate 1% by scattering the powders to a water reservoir and malathion 2.5% for fogging (Laws et al 1968). However, the routine uses of abate and malathion will lead to cause environmental pollution, the death of other living creatures and cause larvae to become resistant, it can even lead to genetic mutations (Ndione et al 2007). Observing the many negative effects caused by synthetic larvicides, it takes an effort to get other alternative materials that are more environmentally friendly, but also effective in eradicating the *A. aegypti* mosquito larvae.

Aceh Province (coordinate 5º33′N 95º19′E) of Indonesia has a vast coastal areas. In the coastal areas overgrown with various kinds of perennial herbs. The herbs have a secondary metabolite that is used to defend itself from pests and predators. Flora have been used by humans in their health management. Humans use it due to the content of floristic compounds thought to have healing properties. Rural communities inhabited the coastal region have also been associated flora in their health activities such as nuisance insect control. *Ipomoea pes-caprae* (L.) R. Br. is one of the plants that grows in coastal areas. The plant contains active compounds of steroids, alkaloids, terpenoids, flavonoids, saponins and tannins (Bandaranayake 2002; Bragadeeswaran et al 2010). This plant is often used in folk and tribal medicines and has various types of biological activities (Premanathan et al 1996; Meira et al 2012). The literature traces informed that there are no scientific studies executed to the larvicidal activity of *I. pes-caprae*. Therefore, this

study was focused to examine the larvicidal potentials present in *I. pes-caprae* parts (roots, stems, leaves, flowers, and seeds) extracted with methanol and water solvents.

The searching of larvicidal candidate from *I. pes-caprae* parts was performed with the following strategy. The plant part of roots, stems, leaves, flowers and seeds was collected. Each part of the plant was divided into two portions (A, and B). The A portion was dried indirectly to sunlight, and then each material of A was blended to get the powder. The powder of each part of A was extracted with methanol solvent. The mixture was filtered. Each part of the methanol filtrate was vacuum evaporated to obtain a crude methanol extract. The B portion, each part of the material was blended to get pulp, and then the pulp was boiled. Each part of the decoction was filtered. Each part of the water filtrate was vacuum evaporated to get a crude water extract. All of obtained extracts were examined their toxicity against the 3rd instar larvae of *A. aegypti*. The criteria applied to select larvicidal candidate were (1) the concentration of the extract solution must be ≤ 50 ppm, and (2) the larval mortality due to administration of the extract should be reached ≥ 75%. The criteria were based on planned to get larvicide that has high toxicity with lower concentration. Extracts met these criteria run to phytochemical screening to identify the secondary metabolite components (Table 1).

Table 1

Phytochemical analysis on the leaves of *I. pes-caprae*

Compound group	*Constituens present in methanol extract*
Saponins	+
Alkaloids	+
Triterpenes/Steroid	+
Flavonoids	+
Tannins	+
Glycosides	+

Note: "+" = detected.

Material and Method

Plant material. All parts of *I. pes-caprae* plant (roots, stems, leaves, flowers, and seeds) were collected from the beach area Krueng Cut village (coordinate 5º35′12″N 95º21′26″E), Aceh Besar District of Aceh Province, Indonesia, as shown in Figure 1. This study was conducted from March to June 2013 in the Laboratory of Marine Chemistry of Syiah Kuala University, Darussalam - Banda Aceh, Indonesia.

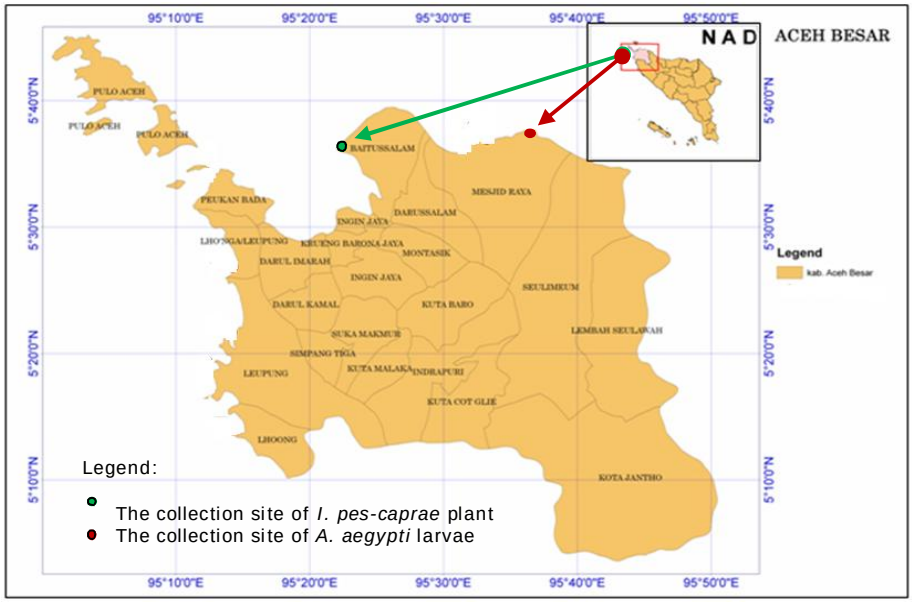

Figure 1. Map of the collection site of the *I. pes-caprae* and *A. aegypti* larvae at the Aceh Besar District of Aceh Province, Indonesia.

Extraction of I. pes-caprae parts in methanol solvent. Any portion of *I. pes-caprae* plant was dried under indirect sunlight for 5 days. The dried material was separately blended to make powder with a sieve size of 40 meshes. The powder was balanced as much as 1, 5, 5, 1, and 1 g for root, stem, leaf, flower, and seed, respectively, and put it into a 100 mL conical flask in volume. 50 mL of absolute methanol was poured slowly into the flask and stirred the powder homogeneously, and then the mixture was left for 24 hours. The mixture was separated through Whatman #1 paper. The filtrate was evaporated over vacuum evaporator to obtain a crude extract of 86, 476, 775, 105, and 98 mg for root, stem, leaf, flower, and seed, respectively. The extracts were kept in the refrigerator for 7 days prior bioassay.

Extraction of I. pes-caprae parts in water solvent. Each portion of the fresh plant as much as 1, 5, 5, 1, and 1 g of root, stem, leaf, flower, and seed was blended to make pulp. Each pulp was boiled with 25 mL water as long as 10 minutes while being stired, and let stand until cool. The mixture was separated through Whatman #1 paper. The filtrate was evaporated over vacuum evaporator to obtain a crude extract of 37, 226, 523, 67, and 56 mg for root, stem, leaf, flower, and seed, respectively. The extracts were kept in the refrigerator for 7 days prior bioassay.

Phytochemical screening. The presence of secondary metabolites such as triterpenes/steroids, alkaloids, flavonoids, tannins, saponins, and glycosides was detected through standard methods (Tiwari et al 2011).

Collection of A. aegypti larvae. The 3rd instar of *A. aegypti* larvae were collected from local water reservoirs Ujung Batee village (coordinate 5º39′2″N 95º25′24″E), Aceh Besar District of Aceh Province, Indonesia (Figure 1). Samples were selected in accordance with the criteria of healthy larvae (Lee 1990). The larvae were placed in a 50 mL beaker glass and labeled it according to the treatment. Each beaker glass was filled with water taken from the larvae habitat as much as 10 mL. The ten larvae were put in to each of the beaker glass. The larvae were acclimatized for 30 minutes to allow them to adapt to the surrounding.

Testing larvicidal candidate of the extracts. Each the methanol and water extracts of the plant parts were weighed as much as 2 mg and dissolved in tap water to a volume of 40 mL to obtain a solution of 50 ppm. Each of the aqueous solution (50 ppm) of the extracts was transferred as much as 10 mL into sterile beaker glasses (50 mL in volume). Ten third instar of *A. aegypti* larvae were separately introduced into different beaker glass containing graded concentration and the larval mortality was recorded after 48 hours since the exposure time. The extracts have not met the criteria, *i.e*. (1) the concentration of the extract solution must be ≤ 50 ppm, and (2) the larval mortality due to administration of the extract should be reached ≥ 75%, should be rejected as the larvicidal candidate of *A. aegypti* larvae (Table 2).

Table 2

Searching of *I. pes-caprae* parts as larvicidal candidate

Part of I. pes-caprae	*The portion of extracts met to the (1) and (2) criteria*	
	methanol extract	*water extract*
roots	no	no
stems	no	no
leaves	yes	no
flowers	no	no
seeds	no	no

Note: (1) the concentration of the extract solution must be ≤ 50 ppm, and (2) the larval mortality due to administration of the extract should be reached ≥ 75%.

Making an aqueous solution of I. pes-caprae leaf methanol extract. The methanol extract of the plant leaf was weighed as much as 6 mg and dissolved in tap water to a

volume of 120 mL to obtain a solution of 50 ppm as the mother liquor. The mother liquor was diluted to obtain a solution of 40, 30, 20, and 10 ppm. Determination of the concentration of these solutions was based on preliminary testing.

Mosquito larvicidal bioassay. The larvicidal bioassay adopted to the World Health Organization standard protocols (WHO 1981). Each of the concentrations of aqueous solution (10, 20, 30, 40, and 50 ppm) of the methanol leaf extract was transferred as much as 10 mL into sterile beaker glasses (50 mL in volume). Ten third instar of *A. aegypti* larvae were separately introduced into different beaker glass containing graded concentrations and the larval mortality was recorded after 48 hours since the exposure time. Larvae were identified dead when the organism failed to move after touching with a needle in the siphon or cervical site. The experiments were run in four replications and conducted under laboratory conditions at 25–30°C and 80–90% relative humidity.

Statistical analysis. The methodology of this study was referred to the completely randomized design (CRD) with six levels of concentration (0, 10, 20, 30, 40, and 50 ppm) and four replications. Mortality data of *A. aegypti* larvae were analyzed using Trimmed Spearman-Karber (TSK) program version 1.5 in order to obtain LC_{50} value (Hamilton et al 1977).

Results and Discussion. In the present study, the toxicity of methanol extract of *I. pes-caprae* leaf on the *A. aegypti* larvae increased with increasing concentration of the solution (Table 3). Observation on the control showed that all of *A. aegypti* larvae were still alive in the period of 48 hour-exposure. There was the death of the *A. aegypti* larvae by 40, 70, and 80% after exposing to the concentrated solutions of 10, 20, and 30 ppm, respectively, while at the concentrations of 40 and 50 ppm, all of *A. aegypti* larvae died. The data showed that increasing the concentration of the methanol extract of *I. pes-caprae* leaf exposed to *A. aegypti* larvae would increase percentage mortality of *A. aegypti* larvae. The increasing concentration of the extract led to increase the content of poisonous active ingredient in the extract that could kill target in large numbers (Kardinan 2005).

Table 3

The effect of *I. pes-caprae* leaf methanol extract on the mortality of *A. aegypti* larvae

Conc. of *I. pes-caprae* extract (ppm)	Number of *A. aegypti* larvae per application (ind)	Mortality of *A. aegypti* larvae (%)
0	10	0
10	10	42.5
20	10	72.5
30	10	77.5
40	10	100
50	10	100
L_{50}, ppm	12.60	
Lower limit, ppm	7.44	
Upper limit, ppm	21.34	

Note: bioassay was conducted for 48 hours, and a level of confidential interval was set at 95%.

Observation on *A. aegypti* larvae exposed to methanol extract of *I. pes-caprae* leaf exhibited early signs such as a rapid mobility up to the water surface and down to the bottom of the beaker glass. This movement was assumed as the initial response of *A. aegypti* larvae against toxic substances originated from the methanol extract of *I. pes-caprae* leaf. The movement of larvae in the tested solutions differed from the movement of larvae in the control, which showed low mobility. The *A. aegypti* larvae exposed to toxic substances revealed a move up and down in the water medium. This mobility was dissimilar movement from that seen in the control (Yunita et al 2009).

Observation to the larval cuticle exposed to the methanol extract of the plant leaf showed a darkening of the larval cuticle compared to the larval cuticle color of the

control. In addition, the body length of the dead larvae performed longer than the larval body length before giving the extract. These supposed due to the present of saponin-containing steroid hormones in the methanol extract of *I. pes-caprae* leaf. The observations were also stated by another study reporting that the larvae fed food containing steroid hormones led to a relaxation of the tendon caused the longer larvae size and larval body color was black (Asiah et al 2009).

Based on the results of the phytochemical screening on the plant leaf extract, the extract contained a compound class of saponins, alkaloids, flavonoids, triterpenes, steroids, glycosides and tannins (Table 1). The secondary metabolites in the plant leaf extract were in line with other investigator report stated that the plant contained steroids, alkaloids, terpenoids, flavonoids, saponins and tannins (de Souza et al 2000). Saponins are glycosidic compounds that are freely soluble in both methanol and water solvents. Saponins interact with the larval cuticle membrane cause damage to the membrane structure, and could be suspected as the reason of death of the larvae (Wiesman & Chapagain 2005). In addition, saponins also have a bitter taste that decrease appetite larvae then larvae will die from starvation (Minarni et al 2013). Other ingredient contained in the leaf extract was tannin. Tannin is a substance that could interfere with the larvae in the process of digestion. Tannins would bind to proteins in the digestive system of larvae necessary for growth, therefore, the absorption of protein in the digestive system becomes disrupted (Boudko et al 2001). The presence of alkaloids in the extracts exposed to the larvae was characterized by larval mobilities such as curling up, agitation, vigorous body movements which were the characteristic of neurotoxicity (Madhumathy et al 2007). The alkaloids could act as a hypoglycemic agent that leads to mortality of the larvae (Sethuraman et al 2010). Secondary metabolites such as alkaloids, steroids, phenolics, triterpenes have good mosquito larvicidal properties (Singha et al 2012). Based on the data analysis of *A. aegypti* larvae mortality for 48 hours by means of a TSK (Trimmed Spearman-Karber) program version 1.5 was obtained a LC_{50} value as much as 12.60 ppm with a lower limit of 7.44 ppm and an upper limit of 21.34 ppm at 95% confidential interval. The methanol extract of *I. pes-caprae* leaf showed highly toxicity, LC_{50} (12.60 ppm) and induce 100% mortality at 40 ppm, compared to the *I. cairica* essential oil, LC_{50} (22.3 ppm) and induce 100% mortality at 120 ppm against *A. aegypti* larvae (Thomas et al 2004). The toxic properties of the *I. pes-caprae* leaf methanol extract could be due to the combined effect of the diverse secondary metabolites in the crude extract and could exhibit a different mode of action towards the larvae causing in high toxicities.

Conclusions. The methanol extract of *I. pes-caprae* leaf could be applied as an antidote in controlling dengue larvae in vases, containers and other dispose of household tools in which this dengue mosquito habitually breed. There has been no information about the larvicidal activity of *I. pes-caprae*, therefore, this is firsthand evidence confirmed *I. pes-caprae* having an excellent potential as larvicidal agent against *A. aegypti*.

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