PERMOT (*PASSIFLORA FOETIDA* LINN.) LEAF EXTRACTS AS BIOINSECTICIDE AGAINST *AEDES AEGYPTI* LARVAE

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Abstract. The aim of this study was to investigate the effectiveness of *Passiflora foetida* Linn. extracted by various organic solvents (n-hexane, ethyl acetate and ethanol) against IV instar *Aedes aegypti* larvae. The components fractionated by thin layer chromatography of n-hexane extract were alkaloids and terpenoids, that of ethyl acetate fraction was terpenoid, and that of ethanol fraction was phenol. Gas chromatography-mass spectrometry analysis of n-hexane fraction indicated that the presence of isophytol, neophytadiene, 9,12,15-octadecatrienoic acid, 13-octadecenal, and phytol; and of the n-hexane fraction as isophytol and phytol. Larvae (n = 25) were exposed to each organic solvent extract (0, 500, 1,000, 1,500, and 2,000 mg/l) for 24 hours, each experiment conducted four times. Mortality data were analyzed using probit analysis at 95% significance level. The 50% lethal concentration of n-hexane extract, 440 mg/l, was the lowest, but too high to be used effectively against *Aedes aegypti* larvae. Further purification will be required to identify the active larvicidal compound(s).

Keywords: *Aedes aegypti, Passiflora foetida,* fourth instar larva, larvicidal activity

INTRODUCTION

Surabaya city, Indonesia is prone to dengue hemorrhagic fever (DHF), with the incidence increasing yearly (Suroso, 1997). The highest incidence of DHF in Surabaya was 58 per 100,000 in 1968, with 24 deaths (a case fatality rate of 41,3% compared to a mean of <1%) (Surabaya City Health Department, 2005). The disease has spread to numerous cities and almost all provinces in Indonesia are affected by 2004 (Soegianto *et al*, 2004).

Dengue is caused by dengue virus transmitted by mosquito vectors *Aedes aegypti* and *Aedes albopictus* (Sumarmo, 2004). The increasing number of dengue virus infections each year is closely related to environmental sanitation, social-economic conditions and human behavior (Arsin and Wahiduddin, 2004). The most appropriate way to cope with this disease is to control the vectors, a key strategy employed around the world (Okumo *et al*, 2007).

The use of insecticides directed at the larval stages of the mosquito is accepted as the most general way to control these insects. The use of Abate SG (1% temephos sand granules) has been employed in Indonesia since 1976 (Suroso, 1997). Four years later, it was established as part of a mass eradication program of *Ae. aegypti* (Ministry of Health Indonesia, 1999). But the danger of resistance and side effects caused

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by insecticides is inevitable, and resistance of *Ae.aegypti* against temephos was found in Surabaya (Rahardjo, 2006). Given the rapid development of insecticide resistance in vector mosquitoes, the development of alternative sources of insecticides, such as those derived from plants (bioinsecticide) is a sound strategy. Replacement of chemical insecticides with more environmentally friendly bioinsecticides is essential to anticipate the negative health impact of DHF. One of the alleged medicinal plants containing active insecticidal compounds, which can be utilized as a bioinsecticide against *Ae. aegypti* is the plant Permot (*Passiflora foetida* Linn.), which grows wild and can be easily obtained in Indonesia (Wijayakusuma *et al*, 1995).

The purpose of this study was to extract, isolate, and analyze chemical components from Permot leaves and to investigate the potential of the extracts as bioinsecticides against *Ae. aegypti* larvae.

MATERIALS AND METHODS

Collection of Permot leaves

Permot leaves were collected from several locations in Surabaya. Identification was performed at the LIPI Purwodadi Botanical Garden, Pasuruan, East Java. Leaves were cleaned and aerated for 5-7 days by placing them in an area sheltered from the sun, and then ground to powder (simplisia).

Rearing of mosquito larvae

Mosquitoes utilized in this study were fourth instar *Ae. aegypti* larvae. Rearing of *Ae. aegypti* eggs were performed by placing them in plastic trays filled with water at 28.5°C and 72.5 % humidity. Eggs hatched after 1-2 days and the first intstar larvae were moved to another tray containing 1 liter of water and fed with four catfish pellets (Comfeed, Pandaan-Pasuruan, East Java, Indonesia). After 1-2 days the first instar larvae developed into second instar larvae, characterized by exfoliation, which then moved to another tray also contains 1 liter of water and catfish pellets. Within 2-3 days third instar larvae appeared and by the following 2-3 days fourth instar larvae were generated.

Extraction of Permot leaves

One kg of Permot leaf was macerated in 1 liter of n-hexane and left to stand for 24 hours. The solution was then filtered and the process was repeated three times. The residue was re-extracted with 1 liter of ethyl acetate and processed as described above. The residue was again re-extracted with 1 liter of ethanol solvent as described above. The filtrate from each solvent was collected and evaporated using a rotary evaporator under reduced pressure to obtain a viscous extract.

Thin layer chromatography (TLC) analysis of n-hexane, ethyl acetate and ethanol extracts

The presence of alkaloids, flavonoids, phenols, and terpenoids in ethanol, ethyl acetate and n-hexane extracts were tested by TLC using aluminium foil-backed silica gel and hexane as mobile phase (followed by treatment with Dragendorf reagent), or a mixture of n-hexane, ethyl acetate (4:1) (treatment with anisaldehyde sulfuric acid), or a mixture of chloroform:ethyl acetate:formic acid (0.5:9:0.5) (treatment with $FeCl_3$.

Identification of chemical compounds by gas chromatography-mass spectrometry (GC-MS)

Fraction containing the lowest LC_{50} value (concentration producing 50% lethality) was analyzed using GC-MS. The sample solution inserted to GC-MS 2010 S

(Shimadzu, Portland, OR). Identification of peaks was performed based on database from LIBRARY WILEY7-LIB.

Determination of LC_{50} value

Ethanol, ethyl acetate and n-hexane extracts were emulsified in distilled water using Tween 80 and dimethyl sulfoxide (4:1 v/v). Fourth instar larvae of *Ae. aegypti* (n = 25) were soaked for 24 hours with each solution of 0, 500, 1,000, 1,500, and 2,000 mg/l. Larvae are scored as killed when they did not move or respond to mechanical stimuli. Each experiment was repeated four times.

Data analysis

Relationship between concentration test solution and mortality of fourth instar *Ae. aegypti* larvae was analyzed using Probit for Windows SPSS 10 (SPSS, Chicago, IL) to obtain lethal LC_{50} values at a significance level of 95%.

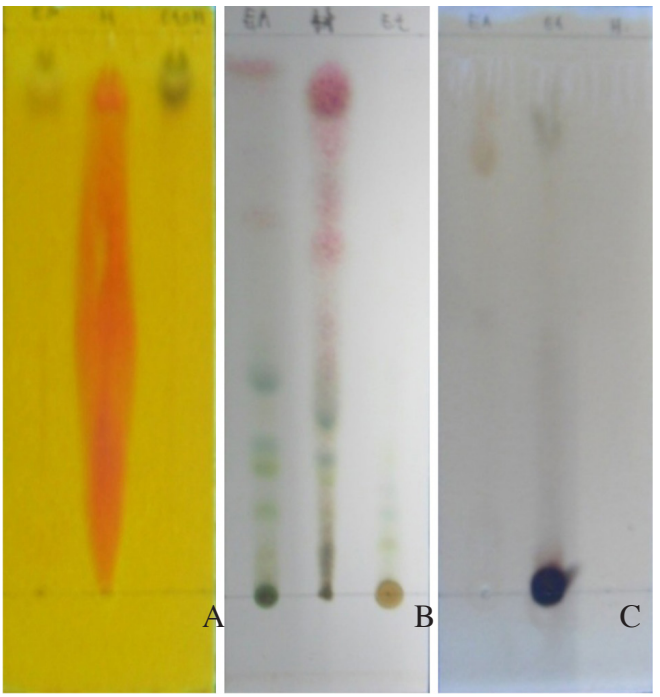

Fig 1–Thin layer chromatograms of Permot leaf ethanol (EOH), ethyl acetate (EA) and n-hexane (H) extracts. A) Separation with hexane and treatment with Dragendorf reagent. B) Separation with a mobile phase of mixture of n-hexane, ethyl acetate (4:1), and treatment with anisaldehyde sulfuric acid. C) Separation with a mobile phase of a mixture of chloroform:ethyl acetate:formic acid (0.5:9:0.5) and treatment with $FeCl_3$.

RESULTS

TLC identification of Permot leaf ethanol, ethyl acetate and n-hexane extracts

One kg of Permot leaf of simplisia yielded 126, 27 and 34 g of ethanol, ethyl acetate and n-hexane extract, respectively, which were separated by TLC. N-hexane extract was positive with Dragendorf reagent (orange red) indicating the presence of alkaloids (Fig 1A). A mobile phase of a mixture of n-hexane:ethyl acetate (4:1) and anisaldehyde sulfuric acid treatment resulted in red purple or violet color indicative of the presence of terpenoids in n-hexane and ethyl acetate fractions (Fig 1B). A mobile phase of a mixture of butanol:glacial acetic acid:water (4:1:5) and treatment with chloroform:acetone:formic acid (6:6:1) under ammonia vapor failed to produce a yellow color in any of the three extracts indicating the absence of flavonoids (data not shown). A mobile phase of a mixture of chloroform:ethyl acetate:formic acid (0.5:9:0.5) and $FeCl_3$ treatment generated a black stain with the ethanol extract indicating the presence of phenols (Fig 1C).

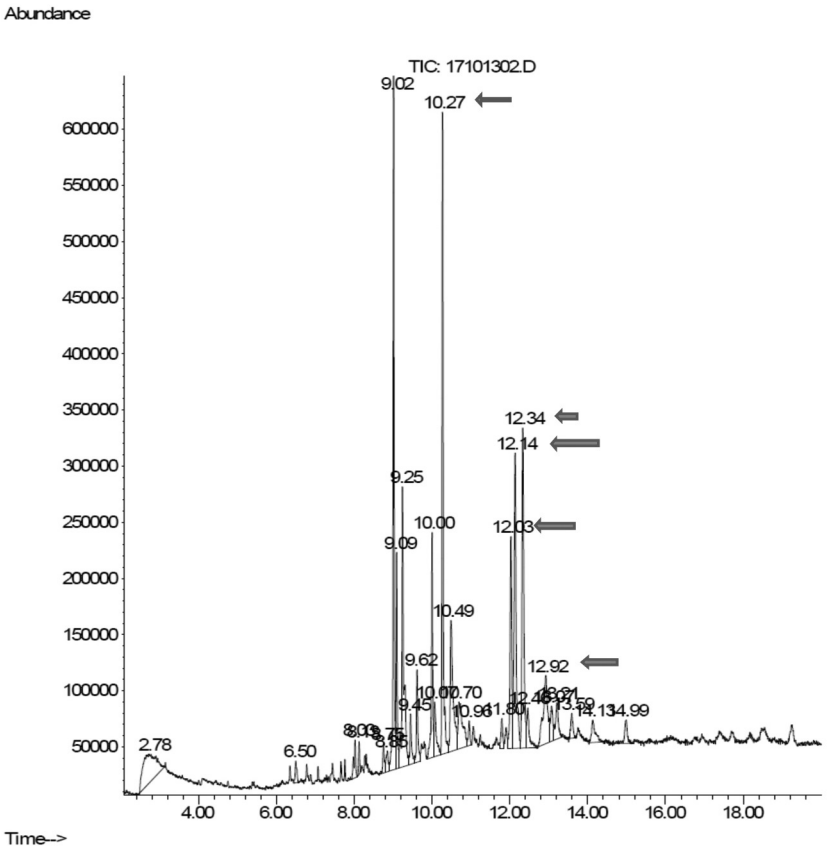

Fig 2–Gas chromatography-mass spectrometry profile of Permot leaf n-hexane extract displayed 28 peaks GC-MS indicated that the n-hexane extract contained alkaloids and terpenoids (isophytol and phytol compound). Five peaks of which were subsequently shown to be isophytol, neophytadiene, 9,12,15-octadecatrienoic acid. GC-MS used for analyzing fraction containing the lowest LC50 value. The sample solution inserted to GC-MS. Based on chromatogram of GC can be known the profile of peak of sample components. Result of quality analysis shown with a lot of peak that represents a lot of compounds. In quantitative analysis the height or peak area represents the relative procentage of the compound. Numbers above peaks shows time retention (in minute).

GC-MS identification of Permot leaf hexane extract

GC-MS analysis of Permot leaf n-hexane extract displayed 28 peaks (Fig 2), five peaks of which were subsequently shown to be isophytol, neophytadiene, 9,12,15-octadecatrienoic acid, 13-octadecenal, and phytol (data not shown).

Toxicity tests of Permot leaf extracts against fourth instar *Ae. aegypti* larvae

Probit analysis of the toxicity of Permot leaf extracts (concentrations ranging from 500 to 2,000 mg/l) against fourth instar *Ae. aegypti* larvae revealed among the three extracts that of n-hexane was the most potent, with an IC_{50} of 440 mg/l (Table 1).

DISCUSSION

Of the three organic solvent extract, namely, ethanol, ethyl acetate and n-hexane, of Permot leaf the latter extract possessed the lowest LC_{50} against fourth instar *Ae. aegypti* larvae. TLC of Permot leaf n-hexane extract indicated that it contained alkaloids and terpenoids. Wijayakusuma *et al* (1995) found alkaloid compounds in Permot plant, which possess the ability to work as a poison against mosquito larvae and are more abundant in leaf than in other parts of the plant. GC-MS further indicated that the n-hexane extract in addition contained isophytol and phytol compounds, both of which are terpenoids, in line with the

Table 1
Probit analysis of LC_{50} and LC_{95} of Permot leaf extracts against fourth instar *Aedes aegypti* larvae after 24 hours exposure.

Extract	LC50 (mg/l)	LC95 (mg/l)	Regression equation
n-hexane	440	1,796	Y = 2.692 X - 7.116
Etthyl acetate	2,122	8,668	Y = 2.692 X - 7.116
Ethanol	3,076	12,560	Y = 2.692 X - 7.116

LC_n = concentration producing n% lethality.

findings of Estrada *et al* (2013) suggesting phytol is the main component of *P. alliaceae* leaf. Mathew and Thoppil (2011) reported that the important components in essential oil of *Salvia splendens* acting as larvacidal against *Ae. albopictus* larvae are phytol and cyclooctasulfur.

Other studies have shown that plant-based insecticide has the power to kill larvae and mosquitoes. For example extract of papaya seeds and leaves have LC50 442,311 mg/l against *Anopheles* sp larvae (Hastuty, 2014). Another study showed larvicidal activity of eucaplitol and camphor from Rosemary leaf extract against *Culex quinquefasciatus* (Yu *et al*, 2013).

Alkaloids possess the ability to work as a good contact poison because of their ability to penetrate the insect cuticle. They are very effective against a variety of insects, especially those with soft-bodies. In larvae, alkaloids work well as a contact poison and stomach poison. These compounds work at the the insect central nervous system ganglia (Soparat, 2010). Alkaloids can also cause gastrointestinal poison by entering through the larvae mouth (Soparat, 2010). However, alkaloids function slowly in insects as they inhibit feeding (a stop-feeding action).

In summary, the study shows that n-hexane extract of Permot leaf was a better biolarvacide against *Ae. aegypti* compared to ethanol or ethyl acetate extract. The n-hexane containing alkaloids and terpenoids (isophytol and phytol). However, the LC_{50} value against *Aedes aegypti* (fourth instar larvae) was considered too low to be used effectively, and future studies should be directed towards isolating and identifying the active components, including toxicological data and effects on non-target organisms and the environment.

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