

Larvicidal Effect of Vinca Fruit Extract (*Vinca rosea*) against *Aedes aegypti* Larvae and Secondary Metabolites Profile by Thin Layer Chromatography

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Abstract

Background: *Vinca rosea* is known contain alkaloids, it was usually used to treat various diseases. Alkaloids from Vinca leaves are also already known have larvicidal activity. Based on this toxicological activity, the fruit of *Vinca rosea* was selected to investigation its larvicidal activity against the 3rd instar larvae of the mosquito vector of dengue haemorrhagic fever (DHF) *Aedes aegypti*. Five concentrations of Vinca fruit extract were tested against the 3rd instar *Aedes aegypti* larvae. The different larval mortality percentages were recorded after 24 hours. Lethal concentration (LC₅₀ and LC₉₀) of Vinca fruit extract were calculated using Probit analysis. Phytochemical compounds of ethanolic extract also investigated using Thin layer Chromatography (TLC). LC₅₀ and LC₉₀ values of fruit extract were 2.987 mg/ml and 32.861 mg/ml. Alkaloids were detected in extract.

Keywords: larvicidal activity, *Vinca rosea*, Chromatography, secondary metabolites, *Aedes aegypti*

Introduction

Aedes aegypti transmit a number of diseases, such as filariasis, chikungunya, and dengue haemorrhagic fever (DHF). DHF causing millions of deaths every year. The only way of reducing the incidence of this disease is by vector control using synthetic insecticide. The synthetic insecticide are more hazardous to handle and not biodegradable. Substances alternative of chemical pesticides, which pollute and threaten future, can be discovered. More than 300 plant species have been reported to have an activity against mosquitoes included Vinca leaves extract (Remia and Logaswany, 2011).

Vinca rosea (Fam. Apocynaceae) is plants grow everywhere in Indonesia (Fig. 1). *Vinca rosea* has more than 120 indol alkaloids and another alkaloids in the whole plant (Van der heijden et al., 2004). The present study was carried out to determine the larvicidal activity of *Vinca rosea* fruit extract against *Aedes aegypti* larvae.

Methodology

Preparation and extraction of plant materials

The fruits of *Vinca rosea* were collected as wild plants at “north Sekip” at Yogyakarta, Indonesia. Fruits were identified at Department of Pharmaceutical Biology Faculty of Pharmacy, UGM Yogyakarta. Voucher specimen was found at this Department. Fruits were washed and dried at 40°C for 12 hours. 370 g dried fruit powder was macerated with 2.5 L of ethanol (96%) for 48 hours. The ethanol was then evaporated to sticky residues. The extract residue was 6.95 % b/b and kept in the refrigerator.



Figure 1. (A) *Vinca rosea* (B) Fruit of *Vinca rosea*.

Bioassay for larvicidal toxicity

Different concentrations of extract (0.4 mg/mL, 0.8 mg/mL, 1.6 mg/mL, 3.2 mg/mL, dan 6.4 mg/mL) were prepared using boiled distilled water. Bioassay were performed according to standard method (WHO, 2005). Twenty five 3rd larvae of *Aedes aegypti* were introduced in different test concentration with a set of control containing distilled water and water without test solution. The glass with the larvae, were kept at room temperature. After 24 hours of exposure, the number of dead larvae were counted to obtained mortality rate. Three replications were maintained for each concentration. Larval mortality were counted after 24 hours.

The LC₅₀ and LC₉₀ were calculated according to Probit methods of Finney (Finney, 1971). The concentrations in probit analysis were transformed to logarithm (Log concentration) and the lethal concentrations (LC₅₀ and LC₉₀) were calculated manually.

Table 1. Results of probit analysis of vinca fruit extracts against larvae of *Ae. Aegypti*.

Extract concentration (mg/mL)	Percentage larval mortality (mean)	Probit value	The linear regression equation	LC ₅₀ (mg/mL)	LC ₉₀ (mg/mL)
0,4	21%	4,19	Y = 1,229X + 4,416 with R = 0,910	2,987	32,861
0,8	21%	4,19			
1,6	27%	4,39			
3,2	44%	4,85			
6,4	76%	5,17			

Results and Discussions

Larvicidal effect of *Vinca* fruit extract

The 24h bioassay is a major tool for evaluating the toxic effect of vinca fruit extract against *Aedes aegypti* larvae. The mosquito larvae exposed under plant extract showed significant behavior changes. The larvae showed restlessness, loss of equilibrium, and led to death. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical Region (WHO, 2005).

In table 1 the 5 concentrations of *Vinca* fruit extract caused larval mortality of 21%, 21%, 27, 44, and 76%. Zero percent of mortality was noted in control. LC₅₀ and LC₉₀ calculated were 2.987 mg/mL and 32.861 mg/mL.

The larvicidal activity shown by *Vinca rosea* is probably due to the presence of the alkaloid which are toxic substances. Phenolic and non-phenolic alkaloids isolated from *Vinca rosea* leaves are known to have toxic effects (Waskito, 1999).

Phytochemical compound of *Vinca* Fruit Extract

Zones with *R_f* 0.33-0.39 gave positive results after being sprayed with

Dragendorff's reagent and gave negative results after being sprayed with Ninhydrin reagent (*R_f* 0.33) That was mean no amino acid spot detected (Fig. 2).

Thin Layer Chromatography

Secondary metabolite compounds of *Vinca* fruit extract was analyzed by Thin Layer Chromatography (TLC) and the spot visualisation was by the spray reagents. Chromatography was performed using aluminium plates precoated with silica gel 60 F₂₅₄ (E.Merck). Extract solution was prepared by dissolving it in ethanol pro analysis. One microliters of the extract solution was applied to the plate. Plates were developed face-down, to a distance of 8 cm, in a glass chamber after conditioning until saturated with mobile phase vapor. Mobile phase that used was ethyl acetate: methanol (9:1 v/v). After development the mobile phase was evaporated to dryness and plates were sprayed with Dragendorff's and ninhydrin reagent.

Positive results with Dragendorff reagent will give orange-red spots or brown with orange background yellow (Wagner and Blatt, 1996).

A positive result with Ninhydrin reagent appeared after heating 95 -120⁰ C about 10 minutes (Jork et al., 1990). Samples containing amino acids will give a positive spots that appear blue-violet.

Tryptophan is the precursor indole alkaloid biosynthesis is a major component of plant alkaloids in *Vinca rosea* (Tikhomiroff and Jolicoeur, 2002). Spraying with Ninhydrin is used to ensure that the zones *R_f* 0.33-0.39 are alkaloids. After isolated brown zones showed the presence of alkaloid compounds in the fruit extract *vinca rosea* (*R_f* 0.33).

The result of *vinca* fruit extract was also proved that they had larvicidal properties against *Aedes aegypti* larvae. Nelson *et al.*, (2006) reported that the whole *V. rosea* plant is poisonous. Whole plant *Vinca rosea* also has a toxic effect on the larvae of *Artemia salina* (brine shrimp) (Gadir, 2012 dan Rahmatullah, 2010). The larvicidal properties exhibited by *vinca* extract in this study might be related to presence of *vinca* alkaloids toxins in the plant.

Conclusion

The present study has demonstrated that an ethanolic extract of *vinca* fruit had capability as larvicides against

Aedes aegypti larvae. Further studies are needed to determine the marker compound for producing Standard Operating Procedure (SOP) and to study their impacts on

human health and non-target organisms in mosquito feeding habitats.

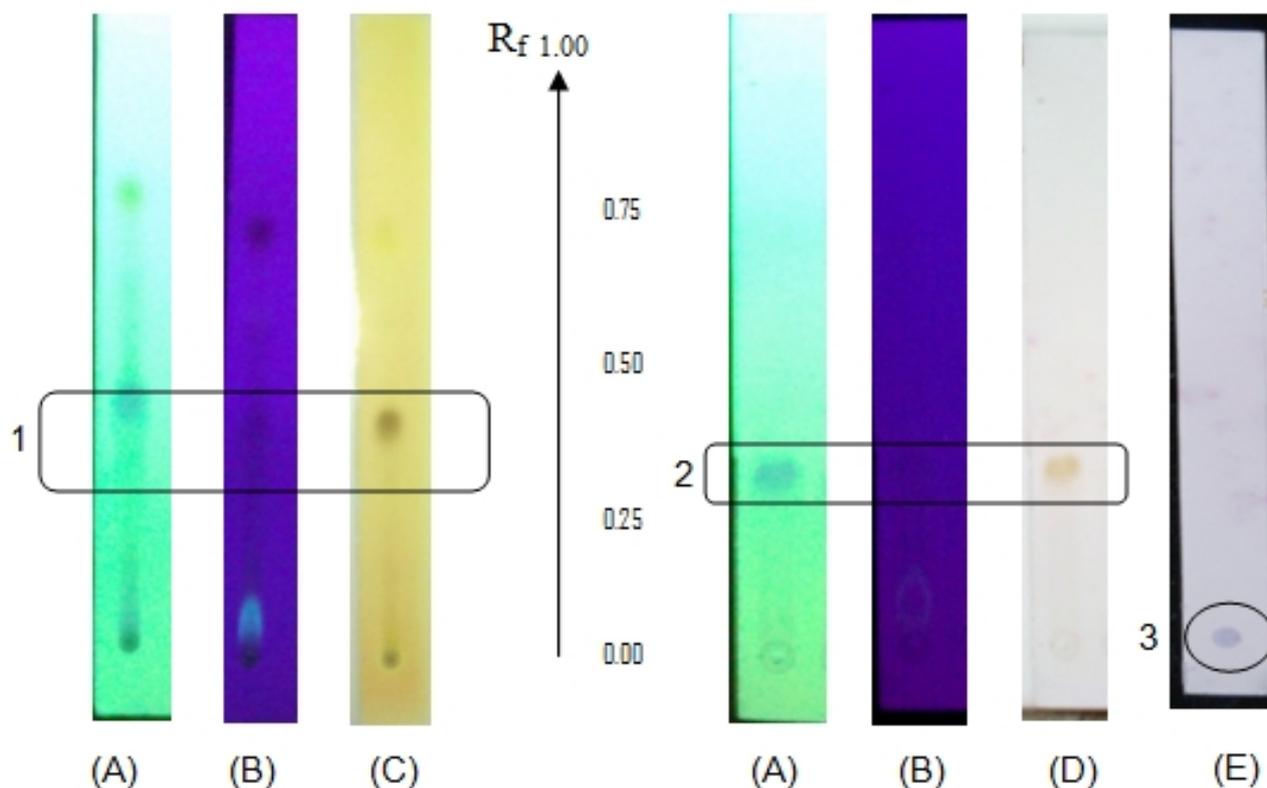


Figure 2. Chromatogram of Vinca fruit ethanolic extract. Solvent systems: ethyl acetate-methanol (9:1 v/v). Detection (A) UV-254nm, (B) UV-365nm, (C) Dragendorff's reagent, (D) ninhydrin reagent, (E) reference for positive result with Ninhydrin reagent: yolk. (1B) alkaloids and another compounds with nitrogen shows fluorescence in UV-365 and (1C) react as brown zones with Dragendorff's reagent ($R_f \sim 0.33-0.39$). (2) Brown zones indicated negative result with ninhydrin reagent ($R_f \sim 0.33$). (3) Amino acids react as blue-violet zones with Ninhydrin reagent.

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