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# Crude methanol extract of brotowali leaves (*Tinospora crispa*) as biolarvacide against dengue vector *Aedes aegypti*

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**ABSTRACT**

This study aimed to determinate potential of crude methanol extract of brotowali leaves (MBL) as biolarvacide against *Aeedes aegypti* larva. Standard WHO bioassay test with slight modification were used. Third instar larva of *Ae. aegypti* were divided into control group and MBL treatment groups (concentrations of 25, 50, 100, 200, 500, 1000, 2000, 5000, 8000 and 10.000 ppm). Mortality was observed after 24 hours of exposure to MBL. MBL at concentration of 200, 500, 1000, 2000, 5000, 8000 and 10.000 ppm produced 6.67, 31.67, 40, 66.67, 98.33, 96.67, 98.33% larval mortality respectively. Larval mortality in the treatment groups were significantly different from control group ($P<0.05$). When the MBL concentrations increased, larval mortality was also raised. Probit analysis revealed that $LC_{50}$ and $LC_{90}$ of this study were 1205.092 and 4104.596 ppm. GC-MS test showed three dominant compounds in MBL, á-Methylphenylethylamine, Benzeneethanamine, and Methyl L-Alaninate. These compounds were classified as amphetamins, alkaloids, and amino acid. Phytochemistry test showed that brotowali leaves extract contained terpenoid. Terpenoid is the compound which usually found in bioinsecticide. Present study indicates that MBL has potential as safer biolarvacides against *Aedes aegypti* larva.

***Key words:*** *Biolarvacides, Aedes aegypti, Tinospora crispa*

## Introduction

Mosquito is well-known vector in transmitting various dangerous diseases (Nazri *et al.*, 2013). *Aedes aegypti* is the main vector in Dengue Hemorragic Fever (DHF) transmission. DHF is endemic in South Asia, The Pasific island area, Africa and America (Park, 2009). Based on WHO data, 390 million DHF infection happen every year and cases reported in more than 100 countries. *Aedes aegypti* is also the transmission vector of chikungunya, lymphatic filariasis, yellow fever and zika virus (Eckert, 2011). The exact way in eradication of dengue disease is by controlling mosquitoes as transmitting vector. Eradication of the *Aedes aegypti* mosquito is often performed using fogging and abate. These methods are not the most effective way because they only kill mosquito, while the larva can still hatch.

*Aedes* habitat is in the clean water. High cases in DHF is due to higher number of breeding places both in urban and rural areas. The use of synthetic insecticides, in the long run, produces negative effects including non-biodegradable chemical insecticides residue, excessive mortality or reduced pro-

ductivity in birds, fish and other non-target organisms and lead to health problem in human (Zaridah *et al*., 2006). Another important issue is mosquito resistance to synthetic insecticides. Besides that, vector control actually can be done in all phases of mosquito life cycle especially larvae phase.

There is a need for alternative methods that are more effective and environmentally friendly than application of insecticides. Plant can be a source of alternative control agent of mosquito and larvae. Biolarvacides from plants are rich in bioactive chemical, active against specific target, provided in massive quantity and biodegradable so it will not contaminate environment (Sukumar *et al*., 1991). Studies of Ishak *et al.* (2014) and Chapagain and Wiesman (2005) reported that *Ipomoea cairica* and *B. aegyptica* extracts could be used as biolarvacide. This study used leaves of brotowali (*Tinospora crispa*). This plant has been used as traditional medicine by Indonesia for years. This plant has anti-inflammation, anti-parasite, antioxidant and antidiabetic activities (Ahmad *et al*., 2016). Brotowali plant may contain secondary metabolites such as alkaloids, terpenoids, lignin, sterols and flavonoids. Other plants which contain this compound are proved to be effective biolarvacide.

However, studies have not been reported on potention of brotowali (*Tinospora crispa*) leaves planted in Indonesia as biolarvacide against *Aedes aegypti*. Therefore, the aim of the present study was to investigate biolarvacide activity of Brotowali leaves against *Aedes aegypti* larvae and to know the bioactive compounds and secondary metabolites in brotowali.

## Material and Method

### Materials and Chemicals

Brotowali leaves (*Tinospora crispa*) were collected from Taman Husada Graha Famili, West Surabaya, Indonesia in November 2018. Methanol pro analys was purchased from Merck (Merck Millipore, Darmstadt, Germany). All other chemicals and solvent used were of analytical reagent grade.

### Preparation of methanol extract from Brotowali leaves

Brotowali leaves (*Tinospora crispa*) (500 g) were cleaned with distilled water, cut into small slices and air-dried for 10-14 days. During air-dried process, brotowali leaves were not allowed to be exposed to sun light and high temperature of oven. Dried brotowali leaves were pulverized using an electric grinder to make fine powder. 168,8 g of dry mass was obtained. Then, the samples were macerated with 700 mL methanol overnight. The extract of brotowali leaves was filtered and macerated twice again with 400 mL methanol. After that, the extract were subjected for rotary evaporatory and freeze-dried to obtain crude methanol extract from brotowali leaves in powdery form.

### GCMS

The crude methanol extract (1 µL) containing different compounds of *Tinospora crispa* was subjected for the Gas cromatography-Mass spectrometry (GC-MS) analysis. GC-MS analysis was performed in PT. Gelora Djaja, Surabaya, Indonesia using Agilent 19091S-433 GC-MS system. The GC-MS system was equipped with HP-5MS 5% Phenyl Methyl Siloxane capillary coloumn (30 m in lenght, 250 µm in diameter, 0,25 µm in film thickness). Helium gas was used as carrier gas. The detector temperature was maintained at 280°C, while the injector temperature was maintained at 300°C. Initial oven temperature was programmed from 150°C, with increase of 2.5°C /minutes to 200°C. Total GC-MS running time was 24 minutes. The detected compounds were identified using data base of National Institute of Standards and Technology (NIST) Library.

### Phytochemical qualitative analysis

Brotowali leaves extract were assessed for the existence of the phytochemical analysis using standard method. The screening was performed for terpenoids/steroid, alkaloids, flavonoids, saponins and tannins. The color intensity or the precipitate formation was used as analytical response to these tests.

### Preparation of *Aedes aegypti* larva

*Aedes aegypti* as vector mosquito larva was used as test organism. *Aedes aegypti* larvae were prepared in Entomology Laboratory, Institute of Tropical Disease, Surabaya, Indonesia. The eggs were soaked in filtered tap water to develop into first instar. Larva feed was administrated 24 hours before placement of egg. Development of larvae to third instars were within three or four days. Third instar larvae were used.

### Larvicidal bioassay

Larvicidal bioassay was performed according to

WHO guidelines (2005) with slight modification. A known amount of brotowali leaves extract (MBL) was dissolved in 95% ethanol to provide stock solution (10.000 ppm). From this stock solution, concentration of 25, 50, 100, 200, 500, 1000, 2000, 5000, 8000 ppm were prepared by dilution. Each concentration was consist of three replications. Test solution (50 mL) was placed in disposable plastic cup and 25 third instar larvae were introduced into the cup. This assay contained three replicate controls which consist of distilled water and ethanol. All cups were kept at room temperature. During the treatment no food was offered to larvae. Mortality was recorded after 24 hours exposure.

**Data analysis**

The concentration lethal to 50% ($LC_{50}$) and 90% ($LC_{90}$) of test organism were determined by probit analysis. Mortalities of treated group was calculated based on Abbott's formula (Abbot, 1925).

$$\% \text{ Mortality} = \frac{\% \text{ kill in treated} - \% \text{ kill in control}}{100 - \% \text{ kill in control}} \times 100$$

One-way analysis of variance (ANOVA) followed by Duncan post hoc test were used to determine the effect of brotowali leaves extract in different concentrations against *Aedes aegypti* larvae. All analysis was performed using IBM SPSS Statistics 24 software. *P*-value of <0.05 was considered statistically significant 850+34.

## Result and Discussion

**Secondary metabolites content based on GC-MS analysis**

Compounds contained in methanol extract of brotowali leaves was presented in Table 1. There were three main secondary metabolite compounds contained in 1000 ppm of brotowali methanol extract. The first compound with the highest percentage was found to be á-Methylphenylethylamine with 68.04% area and 1.421 s retention time. Benzeneethanamine was the second compound with 22.56% area and 1.458 s retention time. The third compound was methyl L-alaninate with 9.41% area and 1.521 s retention time.

**Table 1.** Secondary metabolites content of Brotowali (*Tinospora crispa*) leaves extract based on GC-MS analysis

| Compounds | Area (%) | Retention time (second) |
|---|---|---|
| α-Methylphenylethylamine | 68.04 | 1.421 |
| Benzeneethanamine | 22.56 | 1.458 |
| Methyl L-Alaninate | 9.41 | 1.521 |

These compounds were classified as amphetamins, alkaloids and amino acid. Amphetamine is a central nervous system stimulant that is used in narcolepsy and obesity (Stahl, 2017). Overadministration of amphetamins will lead to abnormal heartbeat, muscle pain, and abnormally fast reflexes (Spiller *et al.*, 2013). Study of Sarwar *et al.* (2015) showed that alkaloids was one of the compounds that responsible for insecticide properties. It could act as contact poison, ingestion or stomach poison, feeding deterrences, and confusant, leading to death of larvae. Amino acid is the compound to from protein. This compound had function for cells signaling and hormone synthesis. However elevated level of amino acid are pathogenic factors for neurological disorders and oxidative stress (Wu, 2009)

**Phytochemical qualitative analysis**

All results of phytochemical analysis are showed in Table 2. Methanol extract of brotowali leaves showed positive result for terpenoids and/or steroids as measured by Thin Layer Chromatography (TLC). The presence of terpenoids and/or steroids was showed by red purple color of spot. Kiesel gel GF 254 was the immobile phase and n-hexane:etyl acetate (4:1) was immobile phase. Phytochemical analysis did not show the presence of alkaloid, flavonoid, polifenol and saponins. Terpenoids and phenylpropanoids can block octopaminem a neurotransmitter in insect, which has similar functions as adrenalin in vertebrates. The phytochemical screening showed that terpenoids were presented in the extract.

**$LC_{50}$ and $LC_{90}$ values**

The result from this study showed that methanol extract of brotowali leaves exhibited larvicidal activity. Probit analysis revealed larvicidal effect against 3rd instar larva with $LC_{50}$ value 120.5.092 ppm and $LC_{90}$ value 4104.596 ppm. From this result, it can be stated that methanol extract of brotowali leaves showed toxic effects so it can be classified as a biolarvacide. According to WHO in 2006, the insecticides is said to be good if it shows high level of

concentration with the shorter amount of time (Haouas *et al.*, 2008). $LC_{50}$ and $LC_{90}$ values of Brotowali extract against larvae of *Aedes aegypti* were presented in Table 3.

**Table 2.** Secondary metabolites content of Brotowali (*Tinospora crispa*) leaves extract based on phytochemical screening

| Metabolites | Result of Phytochemical Screening |
|---|---|
| Alkaloids | +++ |
| Terpenoids/steroids | - |
| Flavonoids | - |
| Polifenol/tannins | - |
| Saponins | - |

+++ : strong intensity reaction; ++ : medium intensity reaction; + : weak intensity reaction; – : non detected.

### Mortality of Aedes aegypti larvae after exposure to Brotowali leaves extract for 24 hours

Dose dependent mortality was observed. After 24 hours exposure, 10 different concentrations of 25, 50, 100, 200, 500, 1000, 2000, 5000, 8000 and 10.000 ppm were tested. Brotowali leaves extract at concentrations of 25, 50 and 100 did not show mortality of larvae. While concentration of 200, 500, 1000, 2000, 5000, 8000 and 10.000 ppm produced 6.67, 31.67, 40, 66.67, 98.33, 96.67, 98.33% larval mortality respectively. Concentrations of 5000, 8000, and 10.000 were significantly different compared to all treatment groups.

**Table 3.** $LC_{50}$ and $LC_{90}$ values of Brotowali extract against larvae of *Aedes aegypti*

| Plant Material | $LC_{50}$ | $LC_{50}$ |
|---|---|---|
| Leaves of Brotowali (*Tinospora crispa*) | 1205.092 | 4104.596 |

$LC_{50}$ = Lethal concentration that kills 50% of the exposed larvae. $LC_{90}$ = Lethal concentration that kills 90% of the exposed larvae.

## Conclusion

We conclude that methanol extract of Brotowali (*Tinospora crispa*) can be used as biolarvacide against larvae of *Aedes aegypti*. This extract had $LC_{50}$ and $LC_{90}$ of 1205.092 and 4104.596 ppm. Concentration of 5000, 8000 and 10.000 ppm produced more than

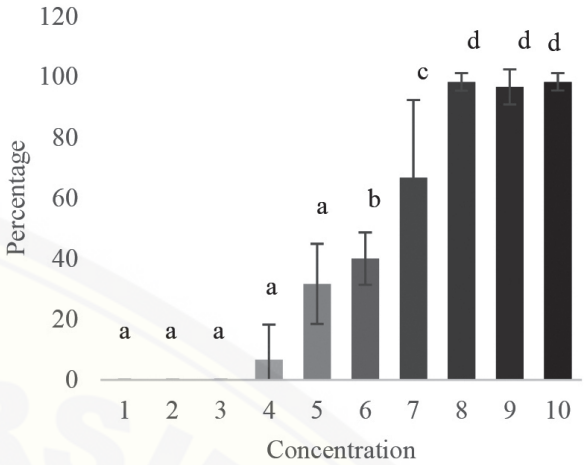


**Fig. 1.** Percentage (%) of dead larva exposed to different concentrations of Brotowali leaves extract. 1: 25 ppm; 2: 50 ppm; 3: 100 ppm; 4: 200 ppm; 5: 500 ppm; 6: 1000 ppm; 7: 2000 ppm; 8: 5000 ppm; 9: 8000 ppm; 10: 10.000 ppm. Each bar represents mean ± SD ($n$ = 25).

90% mortality of larvae. Alkaloids and terpenoids as secondary metabolite compounds contained in the extract may be the bioactive compound to kill larvae. This result made the extract alternative way to replace chemical insecticide.

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