Duku’s Mistletoe Leaves (Dendroptoe pentandra (L.) Miq) Collected from North Sumatera as Botanical Insecticides

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Abstract. Duku’s mistletoe leaves (Dendroptoe pentandra (L.) Miq) is a parasitic plant part with botanical insecticides quality. Preliminary test was done using phenolic extract test with FeCl₃ to know the content of the leaves’s phenolic compound and its toxicity effect. The effects of the toxicity were observed with Brine Shrimp Lethality Test (BSLT) method. D. pentandra are extracted until the total flavonoids are obtained. Test results on its methanolic extract, n-hexane extract, and total flavonoid showed that the extract had cytotoxic potency to shrimp larvae LC₅₀ value of 13.95 µg/ml, 41.59 µg/ml and 7.59 µg/ml respectively. The results showed that the extract of duku’s mistletoe leaves was toxic. These results can be used as a basis to use duku’s mistletoe leaves as an alternative to organo-synthetic insecticides in agricultural product pests control.

1. Introduction
Indonesia is an agricultural country whose people rely heavily on the processing of agricultural products. The problems that faced by farmers today are in the form of pest attacks that can reduce the quality and productivity of agricultural products that will be harvested. Insecticides are needed to control pest attacks but the use of insecticides can endanger human health. Because the synthetic insecticides that commonly used by farmers are toxic [1]. Due to the that reasons, a new type of pesticide that safe for human being is needed to develop.

The use of botanical insecticides is one of the method that able to control pests. Botanical pesticides is produced from plant, it causes this type of pesticide is able to degrade. If this pesticide was consumed by human it will not cause a negative impact because the residue is easily lost and safe [2]. The components of natural products that can be functioned as botanical insecticides are the secondary metabolite, i.e. phenolic, alkaloid, tannin, steroid, etc. In the previous study of abamectin, an insecticide that obtained from the fermentation product of S. avermitilis (Actinomycetes), was able to reduce the level of parasitization and the descendants of Anarus nilaparvatae [3].
Nowadays, many research has been done in the isolation and utilization of secondary metabolite from various plant [4,5]. In the leaves of many plant species, the presence of flavonoid is indicated those plant can be utilized as insecticide, antibacterial, antifungal, and antioxidant [4]. As in the leaves of G. sepium has been utilized to control rice lice [2]. Also, the leaves extract of P. amaryllifolius has been applied as mosquito control [6].

Each bioactive compounds has different properties in the dose toxicity, therefore the in-vivo method, which is called as brine shrimp lethality test (BSLT) can be used as the alternative method to determine the toxicity level of the compounds. One of the organisms that suitable for that in-vivo test is brine shrimp (Artemia salina Leach).

Duku’s mistletoe is usually considered as a parasite on the duku plant and is often not used because it is considered to interfere the growth of duku plants [7]. However, the leaves of duku’s mistletoe are believed to have other benefits such as in controlling pests. Duku’s mistletoe can be easily found in the area of North Sumatra, especially Medan.

Based on the above explanation, the aim of this research is to determine the antioxidant activity of secondary metabolite of duku’s mistletoe leaves. For this reason, the preliminary test is needed to determine the activity of secondary metabolite in duku’s mistletoe leaves as botanical insecticides.

2. Methodology

2.1. Sample
Duku’s mistletoe leaves were collected from Medan, Indonesia

2.2. Preparation of the extracts
Duku’s mistletoe leaf were cleaned, dried for 7 x 24 hours, then were pulverized. The dried leaf powder was weighed (250 g) and macerated using methanol (48 h). The mixture of dried leaf powder was filtered to obtain crude extract. The crude extract was concentrated using a rotary evaporator and water bath. The concentrated extract of methanol was dissolved with water and partitioned with ethyl acetate to separate the tannins. The tannin free extract was dissolved with methanol, then partitioned with n-hexane to separate the nonpolar compounds in order to obtain the total flavonoids compounds.

2.3. Phenolic Test
Five milliliters of methanol extract were placed in a tube and three drops of 1 wt.% FeCl₃ were added. The positive result was indicated by the change in the colour of solution to be black [8].

2.4. Toxicity Test using Brine Shrimp Lethality Test (BSLT) method
Using a spatula about 50 mg of brine shrimp eggs was sprinkled into 500 mL of saline water. The compartment was illuminated through a hole in the lid of the box and kept under a light source using a 40 watts electric bulb. After 48 hours, the phototropic nauplii were collected by use of a Pasteur pipette from the lighted compartment and subjected to brine shrimp lethality test. Ten shrimps were transferred into each of the vial using Pasteur pipette and saline water was added to the 5 ml mark to make dilutions of 1000 μg/ml, 100 μg/ml and 10 μg/ml. three graduated vials were set for each dilution and a further three for the control. The tubes were left at room temperature and the number of live larvae counted after 24 hours. The percentage mortality was determined for each dilution and controls.

3. Results and Discussion

3.1. Phytochemical screening
The result of phytochemical screening test (Table 1) showed the D. pentandra (L.) Miq contained several secondary metabolites, but it was dominated with flavonoid

| No | Secondary metabolites | Reagent          | Result |
|----|-----------------------|------------------|--------|
| 1  | Alkaloid               | Meyer            | -      |
|    |                       | Buchardat        | -      |
|    |                       | Dragendorf/Wagner| -      |
3.2. Toxicity test

The toxicity of methanol, n-hexane extract and the total flavonoid was measured using BSLT method. Table 2 showed the result of BSLT test, the toxicity of total flavonoid is higher than the methanol and n-hexane extracts. The toxicity of D. pentandra (L.) Miq is assumed coming from the quercetine, a flavonoid that commonly found in benalu. A substrate is classified as a toxic if LC$_{50}$ < 1000 µg/ml (for extract) and < 30 µg/ml (for a compound).

| Sample           | Concentration (µg/ml) | Dead value | Total of larvae | Mortality (%) | LC$_{50}$ (µg/ml) |
|------------------|-----------------------|------------|-----------------|---------------|--------------------|
| **Methanol extract** | 1000                  | 28         | 30              | 93.33         | 13.95              |
|                  | 100                   | 22         | 30              | 73.33         |                    |
|                  | 10                    | 17         | 30              | 56.67         |                    |
| **n-hexane extract** | 1000                  | 21         | 30              | 70.00         | 41.59              |
|                  | 100                   | 17         | 30              | 56.67         |                    |
|                  | 10                    | 12         | 30              | 40.00         |                    |
| **Total Flavonoid** | 1000                  | 30         | 30              | 100.00        | 7.59               |
|                  | 100                   | 25         | 30              | 83.33         |                    |
|                  | 10                    | 20         | 30              | 66.67         |                    |
| **Positive control** | 1000                  | 0          | 30              | 0.00          |                    |
|                  | 100                   | 0          | 30              | 0.00          |                    |
|                  | 10                    | 0          | 30              | 0.00          |                    |
| **Negative control** | 1000                  | 0          | 30              | 0.00          |                    |
|                  | 100                   | 0          | 30              | 0.00          |                    |
|                  | 10                    | 0          | 30              | 0.00          |                    |

Note:
- K = Concentration
- LC$_{50}$ = Lethal Concentration 50

![Figure 1. log concentration vs probit % mortality of methanol extract](image)
4. Conclusions
From the toxicity test using the BSLT method it is known that methanol extract, n-hexane extract, and total flavonoids have toxicity potential against shrimp larvae with LC$_{50}$ values of 13.95 µg/ml, 41.59 µg/ml, and 7.59 µg/ml, respectively. This value indicates that the extract of the parasite leaves can be categorized as toxic. This LC$_{50}$ value can be used as a reference to make D. pentandra (L.) Miq extract as an alternative plant-based insecticide for pest control.

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