PRELIMINARY PHYTOCHEMICAL ANALYSIS AND LARVICIDAL ACTIVITY OF EDIBLE FERN (*Diplazium esculentum* (Retz.) Sw.) EXTRACT AGAINST *Culex*

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**Abstract.** Mosquito is one of insects spreading the vector of serious diseases to human being through its bite such as *Culex* which spreads the vector of filariasis and brain inflammatory diseases. Some people have used plants as insecticides traditionally, such as fern (*Diplazium esculentum* (Retz.) Sw., *D. esculentum* (Retz.) Sw. popularly known as edible fern which almost all Asian people, especially Indonesian consumed it as vegetable, and some people also used it for medicinal purposes. This study was performed to investigate the secondary metabolites from *D. esculentum* leaves and its larvicidal activity against *Culex*. The phytochemical screening was undertaken on the leaves and methanol extract of *D. esculentum*, the results showed the presence of alkaloids, steroids, phenols, and tannins on leaves, while methanol extract exhibited existence of steroids, saponins, phenols and tannins. Investigation of larvicidal activity of methanol extract was carried out against *Culex* larvae for various concentrations which observed for 48 hours. Percentage of mortality was analyzed statistically using Bill Test method that showed active larvicidal activity with a LC$_{50}$ value of 149.279 ppm. This study indicates that fern plant have the potential as larvicidal against *Culex* and need to perform the further research to develop the new natural insecticides.

**Keywords:** Phytochemical, larvicidal, insecticide, *Diplazium esculentum*, *Culex*

**I INTRODUCTION**

Mosquitoes are among the insects that pose a serious threat to public health by spreading vector diseases in humans, including malaria, yellow fever, dengue fever and chikungunya [1]. *Aedes aegypti* L., *Anopheles gambiae*, *Culex quinquefasciatus* Say and *Culex pipens* are some of the types of mosquitoes responsible for the transmission of some human diseases caused by protozoa (malaria), nematodes (lymphatics filariasis), meningitis, urticaria and virus (West Nile encephalitis, dengue fever, yellow fever and chikungunya) [2,3]. Today more than 2 billion people in the tropics are the victims of mosquito-borne diseases. Mosquito prevention efforts have been carried out, among others, based on chemical insecticide-based intervention measures [4] and biological control. Mosquito prevention is still focused on the use of chemical insecticides, such as *N*, *N*-diethyl-meta-toluamide (DEET) which commonly found repellent of insects [5]. DEET has remarkable safety profiles after 40 years of widespread use of the world, but toxic reactions can occur, usually when the wrong use of the product [6]. In addition, mosquito spraying also contains DEET which can cause problems to human health, due to direct contact between humans and sprinklers. Besides DEET, organophosphate compound is also used as mosquito prevention formulated in abate. The use of abate as larvicidal is very limited, it is given when an epidemic has occurred. In addition, abate is an inorganic compound that is difficult to decompose in nature, and can cause pests to become immune (pest resurgence). The research of natural materials as mosquito prevention which is easy to obtain, cheap and easy to decompose in nature continues to be developed. Traditionally, some Acehnese people have used plants to overcome mosquitoes, one of the plants used is fern (*Diplazium esculentum* (Retz.) Sw.). Ferns are one of the oldest land plant groups on the surface of our earth. Compared to the other groups, fern plants are usually ignored by researchers, but those are important for the beauty and the economic uses [7]. *D. esculentum* (Retz.) Sw. belonging to Athyriaceae family known as edible fern is a forest plant that has been used by many people since the past as a vegetable. In addition, this plant also has potential of economic, cultural,
ecological and health roles [8]. *D. esculentum* is commonly found growing in humid places such as along the riverbank and also in the forest [9, 10]. Taxonomy of *D. esculentum* are: Kingdom Plantae, Division Tracheophyta, Class Polypodiopsida, Order Polypodiales, Family Athyriaceae, Genus Diplazium Sw, and Species *D. esculentum* (Retz.) Sw. [11,12] (Figure 1).

This plant consists of creeping and branched rhizome; scales brown, lanceolate and up to 7 to 15 mm in length, long fern leaves up to 100 cm and a width up to 20 cm, rather dimorphic, higher fertile leaves, erect, sterile curved. The stalk is green and slightly smooth, with a length of 20 - 50 cm. In leaf 2 - 3 pinnae, 5 - 3 pinnae, and length up to 50 - 80 cm. The pinnules are lancet shaped with a length up to 2 - 5 cm and are somewhat coarsely serrated. The erect stems appear to be fleshy and thick with a length of up to 1.2 m more. Sorus is linear, has a width of 1.5 mm located along the leaf veins, and spores dark brown [13].

**Figure 1 Leaves of *D. esculentum* (Retz.) Sw.**

*D. esculentum* (Retz.) Sw. is widespread in Indonesia such as in Blangkejeren which is one of the sub-districts in Aceh Province. Traditionally, several Blangkejeren's society used it as a natural insecticide by putting the leaves of fern in the corners of the room or in the chicken coop to repel mosquito, flies, and other insects. This plant is very easy to find in Aceh also almost in all regions in Indonesia, both found in the forest and on the edge of rice fields. Several studies reported the traditional use of fern plants as insecticides and repellent of insects and pests [10,13]. Plants are the extraordinary source for the discovery of new medicinal products in drug development [14]. This activity is related to secondary metabolite compounds contained in the plants or also known as natural products [15,16]. Secondary metabolite is a common term used for more than 30,000 different substances which their compounds are usually classified according to their biosynthetic pathways. Three large molecule families are generally considered: phenolics, terpenoids and steroids, and alkaloids [17]. Several studies have reported the secondary metabolite contents in *D. esculentum* [10,18,19,20,21], but fern plants from Indonesia have not been widely reported, even the secondary metabolite contents of Aceh fern has not been reported. Besides, larvicidal activity of *D. esculentum* also has not been reported. Based on those literatures, we report the secondary metabolite content of Aceh fern plant and its larvicidal activity against *Culex*.

**II METHODOLOGY**

**Plant Material and Bioindicator**

*D. esculentum* (Retz.) Sw. leaves were collected from Blangkejeren, Aceh (Indonesia) in February 2018. The bioindicator used in this research is the larva of *Culex*.

**Extraction**

The air-dried leaves (1 kg) of *D. esculentum* (Retz.) Sw. were ground and extracted with methanol by maceration method for 3 x 24 h, the maceration was repeated until the filtrate is clear. The extract solution was filtered and evaporated by rotary evaporator to give methanol extract.

**Phytochemical Screening**

**Alkaloid:** About 2 g of plant materials were crushed then added 1 mL of ammonia. Furthermore, 10 mL of chloroform was added, then crushed and filtered. The filtrate was added 10 mL of sulfuric acid 2N, shaken vigorously, left for a minute until the sulfuric acid solution and chloroform separated. The sulfuric acid layer is taken and divided into three test tubes and each test tube is tested by Meyer, Dragendorff, and Wagner reagents to determine the presence of alkaloids. The addition of Meyer reagent established white precipitate, Dragendorff reagent caused reddish precipitate, and Wagner reagent raised yellow precipitate. Those results indicate the presence of alkaloids [22].

**Terpenoid, Steroid, and Saponin:** Ten grams of fern leaves were finely ground, then extracted with hot methanol. The obtained filtrate was concentrated with rotary evaporator to yield methanol extract. The methanol extract was partitioned with hexane. The soluble extract in hexane was tested with the Liberman-Bourchard reagent. The blue or green color exhibits the presence of steroids and red color for terpenoids. The insoluble residue in hexane is added water.
and shaken vigorously. The presence of the stable foam for 30 min indicates the existence of saponins, if positive for saponins, the solution was hydrolyzed with HCl and tested with the Liberman-Bourchard reagent. The green or blue color indicates the presence of steroidal saponins and the purple or red color shows the existence of terpenoid saponins [23].

**Flavonoid:** Leaves of *D. esculentum* (10 g) was extracted with methanol and concentrated. The concentrated methanol extract was partitioned with hexane. The residue was extracted with 10 mL of 80% ethanol, subsequently added 0.5 mg of magnesium and HCl 0.5 M. The pink or purple color shows the presence of flavonoids [16].

**Phenol:** Methanol extract tested by Ferric Chloride. Add 3 – 4 drops of FeCl₃ solution into extract, the formation of bluish black color exhibits the phenol compound [24].

**Tannin:** About 0.5 g of methanol extract was boiled in 10 ml of water in the test tube and then filtered. Add a few drops of FeCl₃ 0.1%. Forming of a brownish green or bluish black color indicates tannins [25].

**Larvicidal Activity Test**
The mosquito larvae were collected from ditch and identified then incorporated into the cage (50 x 50 x 50 cm) by giving the yeast as food. The larvae were identified before being cultured. The larvicidal test was performed with three repetitions. Methanol extract prepared in test cups for various concentrations of 31.25; 62.50; 125; 250; 500; 1000 and 2000 ppm in water. An abate solution used as positive control and water as a negative control. If the extract is insoluble in water, added dimethyl sulfoxide (DMSO) 0.2% (maximum limit for no effect on the compound activity), the same treatment for positive and negative controls (DMSO amount is converted according to the needs) was performed, stirred until solution is homogeneous. In each test cup, inserted 10 mosquito larvae and incubated for 48 h. After 48 h, the live and dead larvae were counted and analyzed statistically using Bill Test method. The concentration plots with percent mortality to obtain a curve. The obtained curve used to determine the LC₅₀ value.

**III RESULT AND DISCUSSION**

**Phytochemical Screening**
The phytochemical screening was carried out on leaves and methanol extract of *D. esculentum* (Retz.) Sw. using various phytochemical reagents. Examination on leaves showed the active phytochemical classes as alkaloids, steroids, phenols, and tannins, while methanol extract established the presence of steroids, flavonoids, saponins, and tannins as showed in Table 1.

<table>
<thead>
<tr>
<th>Secondary Metabolites</th>
<th>Leaves of <em>D. esculentum</em> (Retz.) Sw.</th>
<th>Methanol Extract of <em>D. esculentum</em> (Retz.) Sw.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Generally, secondary metabolites contained in this plant are polar compounds, those are caused by the solvent used is polar. Although methanol tends to attract almost all different types of polarity compounds, but in this plant material, almost all detected compounds were polar compounds. Analysis on fern leaves indicated the presence of alkaloid compounds which is detected by Meyer reagent that yielded white precipitate, while Dragendorff and Wagner reagents did not show the presence of alkaloids. Even in methanol extract did not exhibited the presence of alkaloids by those three reagents. Examination of terpenoid on leaves and methanol extract showed negative results, but it exhibited the presence of steroids. In the other hand, the presence of saponin compounds was detected in methanol extract but it did not indicate in leaves. The existence of phenols and tannins were showed in both of leaves and methanol extract. Secondary metabolite compounds containing in this plant in general is the phenolic class, namely phenols, and tannins. These compounds contain OH groups which generally have good activity as antioxidant [26,27,28,29]. Several studies have reported the activities of *D. esculentum* (Retz.) Sw. extract such as antibacterial [30,31], antioxidant [10,32,33,34], analgesic [20], hepatoprotective [35], anti-inflammatory [35], and anthelmintic [13]. Traditionally, some of its uses as a medicinal plant were as insecticides and repellent of insects and pests [10,13,20], and to treat measles fever. Compounds that play a role in all those bioactivities are secondary metabolite compounds that contained in the plant of *D. esculentum* (Retz.) Sw.
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Table 2 Bill Test Analysis of Larvicidal Activity

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>Log Dose</th>
<th>Number of Dead Mosquitoes</th>
<th>Number of Living Mosquitoes</th>
<th>Number of Accumulated Deaths</th>
<th>Number of Accumulated Life</th>
<th>Ratio Death : Total</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>3.301</td>
<td>10</td>
<td>0</td>
<td>43</td>
<td>0</td>
<td>1</td>
<td>100.00</td>
</tr>
<tr>
<td>1000</td>
<td>3.000</td>
<td>9</td>
<td>1</td>
<td>33</td>
<td>1</td>
<td>0.9706</td>
<td>97.06</td>
</tr>
<tr>
<td>500</td>
<td>2.699</td>
<td>6</td>
<td>4</td>
<td>24</td>
<td>5</td>
<td>0.8276</td>
<td>82.76</td>
</tr>
<tr>
<td>250</td>
<td>2.398</td>
<td>6</td>
<td>4</td>
<td>18</td>
<td>9</td>
<td>0.6667</td>
<td>66.67</td>
</tr>
<tr>
<td>125</td>
<td>2.097</td>
<td>4</td>
<td>6</td>
<td>12</td>
<td>15</td>
<td>0.4444</td>
<td>44.44</td>
</tr>
<tr>
<td>62.5</td>
<td>1.796</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>21</td>
<td>0.2759</td>
<td>27.59</td>
</tr>
<tr>
<td>31.25</td>
<td>1.495</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>27</td>
<td>0.1290</td>
<td>12.90</td>
</tr>
</tbody>
</table>

Figure 2 Curve of % Mortality vs. Log Dose for Methanol Extract of Diplazium esculentum (Retz.) Sw. Leaves

Larvicidal Activity
In this study, mosquito larvae were taken randomly in nature. Once identified, the mosquito is declared as *Culex*. *Culex* larvae instar 3 or 4 used as bioindicator for larvicidal test. Investigation of larvicidal activity of methanol extract against *Culex* larvae was performed to find out the larvicidal activity of polar compounds from *D. esculentum* leaves against *Culex* mosquito larvae. Larvicidal activity test on methanol extract was carried out at various concentrations of 31.25; 62.5; 125; 250; 500; 1000 and 2000 ppm, by doing three repetitions at each concentration. The results were tabulated in Table 2. The data in table were analyzed statistically using Bill Test method to obtain percent of mortality. Then mortality data is plotted with log dose to determine LC50 (Figure 2). The results showed a strong activity with LC30 value of 149.279 ppm. It showed that methanol extract of *D. esculentum* leaves has good potential as a larvicidal agent. There are several studies that reported the larvicidal activity of some plants on mosquito larvae, such as *Monstera adansonii* plant extracts against *Culex quinquefasciatus* [36], *Nerium oleander* L. flower extracts against *C. quinquefasciatus* Say [37], Xerophytic plants extract against *Culex pipiens* and *Aedes caspius* [38], and pure compounds of Stereum plant against *Aedes aegypti* (Linn) [39]. However, the larvicidal activity of *D. esculentum* extract in this study is the first report. Bioactivity in plants is contributed by the secondary metabolite compounds contained in the plants [40].

CONCLUSION
The phytochemical screening was performed on the leaves and methanol extract of *D. esculentum*, the leaves showed the presence of alkaloids, steroids, phenols, and tannins, while methanol extract exhibited the existence of steroids, saponins, phenols, and tannins. This plant showed potential larvicidal activity against *Culex* with LC30 value of 149.279 ppm. This is the first report about secondary metabolites and larvicidal activity of *D. esculentum* (Retz.) Sw. leaves. It is expected to carry out further research for biological activity of this plant to develop the new natural insecticides.

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REFERENCE


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