Identification of Natural Extracts Secondary Metabolites of Kelakai Leaves *(Stenochlaena palustris (burm.f.) Bedd.)* which Have Potential as Larvicide

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Abstract. Dengue Hemorrhagic Fever (DHF) has always been a serious problem in various regions in Indonesia. This disease is transmitted by the mosquito vector (*A. aegypti*). An effort to overcome DHF is to control vectors using larvacide. However, several studies have shown the vulnerability of mosquito larvae to chemical larvicide (temephos). Therefore, it is necessary to have natural larvicide that are safe, inexpensive, and effective. One of Kalimantan's biodiversity which has the potential as larvacide is Kalakai *(Stenochlaena palustris (burm.f.) Bedd.)*. The method in this study was a phytochemical screening method to detect the content of secondary metabolites such as alkaloids, flavonoids, steroids/terpenoids, saponins, and tannins. The results of phytochemical screening the ethanolic extract of Kelakai leaves showed that it contained flavonoids, tannins, and alkaloid. Whereas for alkaloid and triterpenoid compounds not found in it. Based on reference studies, it is known that positive contain of Kalakai ethanol extract has the potential as larvicide.

Keywords: Kelakai, larvacide, phytochemical, secondary metabolites

1 Introduction

Kelakai (Stenochlaena palustris (burm.f.) Bedd.) Is a type of fern that is commonly found in the forests of Borneo so that it is made as a typical plant of Borneo. Kelakai (Stenochlaena palustris (burm.f.) Bedd.) is a plant that is easy and quick to adapt to nature so that it can grow anywhere, such as tree trunks, rotten wood or dry land. However, Kelakai (Stenochlaena palustris (burm.f.) Bedd.) Will flourish on peat soils because there is quite a lot of water intensity [1].

During this time, Kelakai (Stenochlaena palustris (burm.f.) Bedd.) Has been used by the community for consumption as a vegetable or as a medicinal plant [2]. This is because these plants contain compounds that have a positive impact and are likely to have potential as natural larvicides for Aedes aegypti mosquitoes that can be done as an effort to control dengue vector.

Dengue fever is an acute disease caused by dengue virus. This disease is found in the tropics and sub-tropics and infects widely in many countries in Southeast Asia. The main program data

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of Dengue Hemorrhagic Fever (DHF) in Indonesia which is attached by the Indonesian Ministry of Health states that DHF is still the most cases in Indonesia. Until mid-2018 dengue cases occurred in 34 provinces with 16,940 people and 120 of them died [3]. This also happened in South Kalimantan Province.

Data from the South Kalimantan Provincial Health Office shows that dengue fever is still a serious problem in South Kalimantan Province. This is proven by the occurrence of 103 cases of DHF throughout February 2018. South Kalimantan Province ranks second most DHF sufferers after Central Kalimantan on the island of Kalimantan. In 2018, there were 804 patients with South Kalimantan DHF cases and 29 of them died [4].

The spread of DHF is carried out by the Aedes aegypti mosquito vector, one of which is a breeding ground in a water reservoir. Hidayah research [5] found that the characteristics of potential Aedes aegypti mosquito breeding sites (dark in color, located outside the house, no cover, and well water sources) proved to be related to the presence of larvae. In addition, in previous studies it was found that water parameters (temperature, salinity, and dissolved oxygen) in endemic areas proved to be significantly different and had more potential as a vector breeding ground than in non-endemic areas [6].

Efforts to control the vector of DHF disease so far have been using chemical larvicides, namely temefos powder or commonly known as abate. However, several studies have proven the resistance of mosquito larvae to the Abate powder. Reports of larval resistance Ae. Aegypti has been found in several countries such as Brazil, Venezuela, French Polynesia, the Caribbean and Cuba. Abate resistance in the Southeast Asian region has also been reported, namely in Malaysia and Cambodia [7].

The occurrence of abate resistance in Indonesia has also been found in several regions. As the results of the study of Ponlawat [8] found that there was Aedes aegypti larvae resistance in Surabaya and the results of Istiani research [9] found that abate resistance had occurred in the West Kalimantan and South Kalimantan regions. Based on this, larvicide alternatives are cheap and easily available to the public naturally. The larvicide uses a plant extract, which in this case, Kelakai (Stenochlaena palustris (burm.f.) Bedd.) Is suspected of having larvicidal potential.

Before further research on the potential of Kelakai (Stenochlaena palustris (burm.f.) Bedd.) As larvaside, it is necessary to carry out a phytochemical test of the Kelakai extract so that later this study can be followed up by proving the effect of secondary metabolites contained in Kelakai which can kill larvae. The feasibility extract is thought to be able to be used as larvicide due to the presence of ethyl acetate extract which can kill larvae. Based on this, it is necessary to conduct research on the identification of secondary metabolites contained in the Kelakai extract (Stenochlaena palustris (burm.f.) Bedd.).

2 Method

2.1 Research's Design

This research was conducted in an observational descriptive manner which only observed and identified the contents contained in the Kelakai extract (Stenochlaena palustris (burm.f.) Bedd.) Then the results obtained were described according to the contents in the Kelakai (Fig. 1).

Fig 1. Kelakai (*Stenochlaena palustris (burm.f.)Bedd*.)

2.2 Tempat Penelitian

The study was conducted at the Microbiology and Natural Materials Laboratory of Sari Mulia University.

2.3 Alat dan Bahan

The tools used in this study were measuring cups, chemical digital scales, blenders, plant scissors, dropper pipettes, funnels, petridish, bottles, and filter paper. The materials used in this study were Kelakai (Stenochlaena palustris (burm.f.) Bedd.), Aquades, ethanol, sulfuric acid, sodium hydroxide, hydrochloric acid, chloroforn, Mayer reagent, Wagner reagent and Iron (III) chloride.

2.4 Research's Procedure

2.4.1 The Extraction

Extraction was done by maceration using ethanol. Approximately 1000 g Kelakai (Stenochlaena palustris (burm.f.) Bedd.) Which has been aerated to dry, soaked with 500 mL ethanol, covered and then stored in a dark room for 1 week. After that, the filtrate is removed and the residue is removed (Fig. 2) [10].

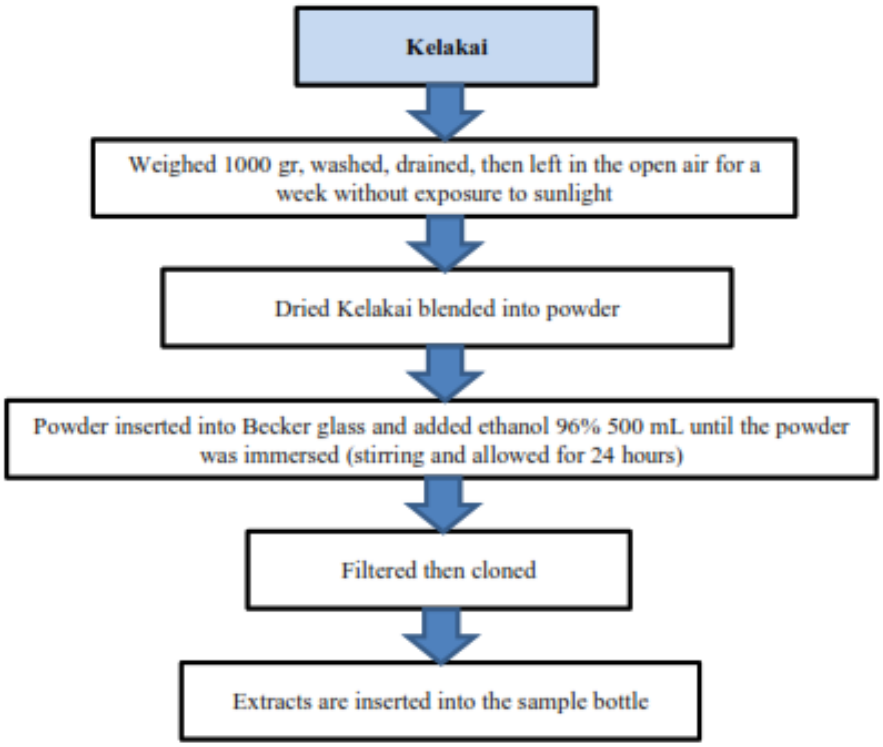


Fig 2. Kelakai (*Stenochlaena palustris (burm.f.)Bedd*.) Extract Processing Flow

2.4.2 Alkaloid Test

The extract sample was dissolved in 2 mL hydrochloric acid, heated 5 minutes, and filtered. The filtrate obtained is added 2-3 drops of Dragendorff reagent. The presence of alkaloid compounds is indicated by orange deposits [10].

2.4.3 Flavanoid Test

A total of 2 mL of sample was dissolved in 2 mL of methanol, then 5 drops of Mg powder and concentrated HCl were added. The presence of flavonoid compounds is indicated by the formation of red or orange [10].

1) Saponin Test

   Saponins can be detected by foam testing in hot water. The foam is stable for 10 minutes and does not disappear with the addition of 1 drop of HCl 2 N showing positive saponin [11].

2) Steroid/Terpenoid Test

   A total of 5 mL of sample was put into a beaker, then chloroform was added and stirred. Next added concentrated H_2SO_4 reagents. If red is formed, it indicates the presence of steroids/terpenoids [12].

3) Tannin Test

   Kelakai extract 10% gelatin drops. It was stated positively contained tannin when it formed a white precipitate or a change in color became turbid [13].

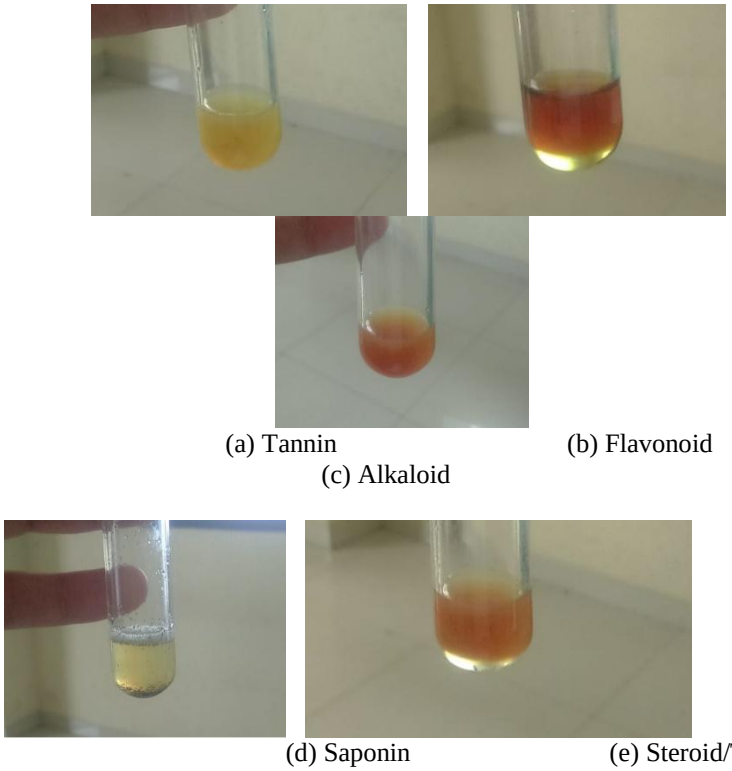
3 Result

The results of the secondary metabolite test of Kelakai extract (Stenochlaena palustris (burm.f.) Bedd.) Can be seen in table 1 and figure 3. Positive results are on flavonoid, tannins, and alkaloids.

Table 1. Kelakai (*Stenochlaena palustris (burm.f.)Bedd.*) Extract Secondary Metabolite Test Results

Type of Compound	Result (+/-)
Flavonoid	+
Tannin	+
Alkaloid	+
Steroid/Terpenoid	-
Saponin	-

(+) = contains secondary metabolites
(-) = does not contain secondary metabolites



(a) Tannin (b) Flavonoid
(c) Alkaloid
(d) Saponin (e) Steroid/Terpenoid

Fig 3. Secondary Metabolite Test Results of Kelakai (*Stenochlaena palustris (burm.f.)Bedd*.) Extract

4 Discussion

Phytochemistry is a preliminary analysis method to examine the content of chemical compounds in plants [14]. Making extracts was carried out with the maseri stage before the phytochemical test was performed. Maceration in the form of powder aims to expand the surface so that the interaction of the solvent with the compound to be taken is more effective and the compound can be extracted perfectly. The smaller the size of the material used, the wider the area of contact between the material and the solvent. This condition will cause the speed to achieve greater system equilibrium. Tissue material or simplicia can affect the effectiveness of extraction [15].

The appropriate size of the material will make the extraction process take place properly and does not take a long time. Periodic stirring aims to avoid compacting the powder so that the solvent is difficult to penetrate the material and it is difficult to take active compounds because the powder used is quite a lot [15].

The flavonoid test was carried out by dissolving the extract in methanol then adding mg and HCl powder. Samples showing positive results contain flavonoids, this is because these flavonoid compounds are more soluble in polar solvents such as methanol. Flavonoids are

compounds that have α-benzopyron nuclei. Oxygen in the carbonyl group will be protected when reacted with HCl. The result of the reaction is a deep red flavilium salt. Alkaloids test showed positive results with the formation of orange deposits [16,17].

The saponin test did not show positive results because the froth formed after shaking did not last long, only lasting a few seconds. Saponins have glycosyl as polar groups and steroid or triterpenoid groups as nonpolar groups so that they are surface active and form micelles when shaken with water. In the micellar structure the polar group faces outward while the nonpolar group faces inward and this is what looks like foam. Steroid and steroid tests also show negative results [16].

The tannin test results are positive or contain tannin compounds. Tannins work by depositing protein and can damage cell membranes so that mold growth is inhibited. Tannin compounds are organic compounds that actively inhibit the growth of microbes by damaging microbial cell walls and forming bonds with functional proteins of microbial cells [15].

The content of these metabolites can be used to kill mosquito larvae, one of which is Aedes aegypti. Several studies have proven this. As the results of Jawale's research [18] it was found that plants containing alkaloids and flavonoids proved to be effective in killing mosquitoes.

According to Suasanto in Hidayah [18], alkaloid and flavonoid compounds can be used as antilarvasides which work similarly to temephos (abate). If the alkaloid and flavonoid compounds enter the body of the larva, the digestive apparatus will be disrupted.

In addition to flavonoid and alkaloid compounds, tannin also has the potential to kill mosquito larvae. Tannins can inhibit the taste receptors in the mouth area of the larvae. This results in larvae not getting a taste stimulus so they are unable to recognize their food and eventually the larvae starve to death [19]. Based on this, the secondary metabolites contained in Kelakai (Stenochlaena palustris (burm.f.) Bedd.) Such as flavonoids, alkaloids, and tannins can make Kelakai (Stenochlaena palustris (burm.f.) Bedd.) As natural larvasides. to kill Aedes aegypti larvae and further research is needed related to this.

5 Conclusion

The results of phytochemical screening the ethanolic extract of Kelakai leaves showed that it contained flavonoids, tannins, and saponins. Whereas for alkaloid and triterpenoid compounds not found in it. Based on reference studies, it is known that positive contain of Kelakai ethanol extract has the potential as larvicide.

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