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Larvacidal And *Oviposition Deterrent* Activity of Dragon Scale Fern (*Pyrrosia piloselloides* (L.) M. G. Price) Extract Against *Culex sp.*

Uji Aktivitas Larvasida dan *Oviposition Deterrent* Ekstrak Paku Sisik Naga (*Pyrrosia piloselloides* (L.) M. G. Price) terhadap Nyamuk *Culex sp.*

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ABSTRACT

Dragon scale fern (Pyrrosia piloselloides (L.) M. G. Price) is one of the plants that grows and spreads wild throughout the tropics and this plant is an epiphyte (a plant that attaches to the stems of other plants), but is not a parasite because this plant can make its own food. This study aims to test the larvicidal and oviposition deterrent activities of the methanol extract of dragon scale fern. Dry leaves of P. piloselloides (L.) M. G. Price were macerated with methanol to produce 30.5 g of methanol extract. Methanol extract contained secondary metabolites compounds of steroid, saponin, flavonoid, and tannin. The methanol extract was tested for larvicidal and oviposition deterrent activities against Culex sp. mosquitoes with concentrations of 31.25; 62.50; 125; 250; 500; 1000; and 2000 ppm. The results showed strong larvicidal activity (toxic) with an LC_{50} value of 143 ppm, and oviposition deterrent activity produces % ER of 72% at a concentration of 2000 ppm. The results of the study showed that methanol extract of dragon scale fern can be used as a natural larvicidal and oviposition deterrent, this activity is contributed by the presence of secondary metabolite compounds, especially flavonoids, saponins, tannins, and steroids.

Keywords: Dragon scale fern (*P. piloselloides* (L.) M. G. Price), mosquito larvae, oviposition deterrent.

ABSTRAK

Paku sisik naga (Pyrrosia piloselloides (L.) M. G. Price) adalah salah satu tumbuhan yang tumbuh dan tersebar secara liar di seluruh daerah tropis dan tumbuhan ini merupakan tumbuhan epifit (tumbuhan yang menempel pada batang tumbuhan lain), tapi bukan parasit karena tumbuhan ini dapat membuat makannya sendiri. Penelitian ini bertujuan untuk menguji aktivitas larvasida dan oviposition deterrent dari ekstrak metanol paku sisik naga. Daun kering P. piloselloides (L.) M. G. Price dimaserasi dengan metanol menghasilkan ekstrak metanol sebanyak 30,5 g. Ekstrak metanol mengandung senyawa metabolit sekunder golongan steroid, saponin, flavonoid, dan tanin. Ekstrak metanol diuji aktivitas larvasida dan oviposition deterrent terhadap nyamuk Culex sp. dengan konsentrasi 31,25; 62,50; 125; 250; 500; 1000; dan 2000 ppm. Hasil pengujian diperoleh aktivitas larvasida yang kuat (toksik) dengan nilai LC_{50} 143 ppm, dan aktivitas oviposition deterrent menghasilkan % ER sebesar 72% pada konsentrasi 2000 ppm. Hasil penelitian menunjukkan bahwa ekstrak metanol paku sisik naga dapat dijadikan sebagai larvasida dan oviposition deterrent alami, aktivitas tersebut dikontribusi oleh adanya kandungan senyawa metabolit sekunder, khususnya golongan flavonoid, saponin, tanin, dan steroid.

Keywords: Paku sisik naga (*P. piloselloides* (L.) M. G. Price), larvasida nyamuk, *oviposition deterrent*

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1. INTRODUCTION

Indonesia is one of the world's richest countries in terms of biodiversity. Sources of biodiversity in Indonesia play an important role in all communities. People, especially in rural areas, tend to use plants as traditional medicines to treat diseases. Data show that there are about 30,000 species of flora in Indonesia's tropical forests, and about 9,600 plant species are known to have medicinal benefits (Nola at al, 2021).

One type of plant that has been used as a traditional medicine is the dragon scale fern (*Pyrrosia piloselloides* (L.) M.G. *Prece*). This epiphytic plant is often found attached to the stems of plants. Although this plant appears to be of no value, dragon scale fern has functions and properties as a medicinal plant, both fresh and dried. This plant is used to treat various ailments such as gingivitis, mouth ulcers, rheumatism, tuberculosis (TB) glands (scrofula) and breast cancer (Kamal, Prayitno and Hamdi, 2021). Dragon scale fern produces various bioactive compounds that can be used as antibacterial, antipyretic, anticancer and antioxidant (Asiandu, Widjajanti and Nurnawati, 2019). Dragon scale fern belongs to the *Polypodiaceae* family. Several studies have reported that plants belonging to the *Polypodiaceae* family have larvicidal activity, such as the *Drynaria quercifolia* plant used in synthesizing nanoparticles have larvicidal activity against *Culex quinquefasciatus* mosquitoes with an LC50 (Lethal Concentration 50) value of 130.725 ppm (Kalaimurgan et al., 2023). Santos et al., 2023 reported that the *Microgramma vacciniifolia* plant is a family of *Polypodiaceae* and stated that the extract from the plant showed larvicidal potential against *Alphitobius diaperinus* larvae. In addition, other plants of the *Polypodiaceae* family have larvicidal and pupicidal effects, namely ethanol extract of the roots and rhizomes of *Dryopteris filix-mas* against the third instar larvae of *Corcyra cephalonica*, where *D. filix-mas* extract 0.20% (v/w) can cause 100% larval death (Shukla, 2011). According to plant taxonomy, the same family has similar chemical content, so it is thought to have the same biological activity (Sukaeningsih, Sukandar and Qowiyyah, 2021).

Larvicide is a method of mosquito larvicide control that is often used by the community because it has several advantages, namely rapid results, ease of use, effectiveness, availability and low cost (Yuliana et al., 2021). Methods to control the spread of mosquito larvae can be biological, chemical and physical. Synthetic larvicides are usually used for larval control because they are very effective, relatively cheap, easy and convenient to use. One of the synthetic larvicides commonly used in the Community is 1% Abate (*Temephos*). However, the continuous use of abate has very dangerous effects, one of which is the contamination of water, especially drinking water. The many negative effects of synthetic larvicides have limited their use. Therefore, the development of natural larvicides is needed to reduce the negative effects of synthetic larvicides (Ekayani et al., 2021). The compounds that have potential as larvicides are flavonoids, saponins, tannins, alkaloids and steroids (Tutik and Marcellia, 2021; Melita, Elsyana and Ulfa, 2022).

In addition to larvicides, oviposition deterrents are also used to control mosquitoes. Oviposition deterrent is a method of inhibiting egg deposition by using chemical compounds to prevent egg deposition. Inhibition of egg deposition provides valuable information that can be manipulated to manage the population.

Mosquitoes are among the animals that become vectors of various diseases. Culex sp. mosquitoes are known as a type of mosquito vector known as the house mosquito. Some types of diseases that can be transmitted by Culex mosquitoes are Japanese Encephalitis (JE) or called an inflammatory brain disease that affects animals and humans, Louis Encephalitis, a disease that attacks the central

nervous system in humans and lymphatic filariasis or called elephantiasis (Hestiningsih et al., 2019).

Based on the above literature, there is a need to develop a natural mosquito control method against Culex sp. Today, no research has been conducted on the use of dragon scale fern for larvicidal and oviposition deterrent testing. However, there are references to plants in the *Polypodiaceae* family having anti-mosquito activity. Therefore, dragon scale fern also has the potential to act as larvicides and oviposition deterrents to control mosquitoes such as Culex sp.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study are aquadest, sugar solution, 98% methanol (CH_3OH), ethyl acetate ($C_4H_8O_2$), n-hexane (C_6H_{14}), dimethylsulfoxide (DMSO), ammonia (NH_3), chloroform ($CHCl_3$), sulfuric acid (H_2SO_4), ferric chloride ($FeCl_3$), Mayer reagent, Dragendorff reagent, Wagner reagent, Lieberman-Burchard reagent (CH_3COOH and H_2SO_4), hydrochloric acid (HCl), ethanol (C_2H_5OH), 10% NaOH solution, mice and Abate solution. The tools used in this research are gloves, masks, glass beakers, Erlenmeyer flasks, filter paper, spatulas, glass jars, beakers, test tubes, tube racks, dropping pipettes, analytical balances, rotary evaporators, funnels, plastic pipettes, aluminium foil, measuring cups, plastic wrap, knives, plastic beakers, watch glasses, gauze, plastic tubs, label paper, mosquito cages, drip plates and canister.

2.2. Sample Preparation and Extraction

A total of 500 g of dried and cut dragon scale fern (*P. piloselloides* (L.) M. G. Price) underwent extraction through a multistage maceration process utilizing n-hexane, ethyl acetate, and methanol as solvents. The maceration was conducted over three cycles of 72 hours each and continued until the filtrate became clear (Halimatussakdiah, Amna and Wahyuningsih, 2018). The resulting extract solutions were filtered and concentrated using a rotary evaporator to obtain n-hexane, ethyl acetate, and methanol extracts. In this study, the methanol extract was used.

2.3. Phytochemical Test

Phytochemical test is one way of identifying the content of secondary metabolites in a natural material. Phytochemical tests were carried out to identify compounds of the alkaloid, terpenoid, steroid, saponin, phenolic, flavonoid and tannin groups (Vifta and Advistasari, 2018). Phytochemical test carried out on alkaloids, terpenoids, and steroids, saponins, phenolics, and tannins in the manner as reported by Halimatussakdiah, Amna and Wahyuningsih, 2018, and a flavonoid test was carried out using the method reported by Kusnadi and Devi, 2017.

2.4. Larvicidal Activity Test

The larvicidal test was carried out with 3 (three) replicates, the methanol extract was prepared in test dishes for different concentrations of 31.25; 62.50; 125; 250; 500; 1000; and 2000 ppm in aquadest. Each treatment included 10 mosquito larvae and was incubated for 48 hours. After 48 hours, live and dead mosquito larvae were counted and statistically analyzed using the Bill test method. The concentration was plotted against percent mortality to obtain a curve, and the curve obtained was used to determine the Lethal Concentration 50 (LC50) value (Halimatussakdiah, Amna and Wahyuningsih, 2018). Larvicides can be calculated as percent mortality using the formula below:

$$\%Mortality = \frac{\text{accumulated number of dead larvae}}{\text{accumulated total number}} \times 100\%$$

2.5. Oviposition Deterrent Test

Synthesis of activated carbon composite the *oviposition deterrent* experiment was conducted using *Culex sp*. mosquitoes initially housed in mosquito cages and subsequently transferred to individual cages measuring 60 cm x 50 cm x 50 cm. These cages were constructed from wood, enclosed with mosquito nets, and equipped with a cloth-covered front panel for access. Within each cage, nine plastic bowls were arranged: seven

bowls containing varying concentrations of extract solutions, one bowl serving as a positive control with temephos, and the other as a negative control. The tested concentrations were 31.25 ppm, 62.50 ppm, 125 ppm, 250 ppm, 500 ppm, 1000 ppm, and 2000 ppm, with each concentration tested in triplicate. Adult mosquitoes were provided with a 10% sugar solution as food, and occasionally, a mouse was introduced as a protein source to facilitate egg production. The experiment was conducted at room temperature over 48 hours, after which the number of eggs in each bowl was counted and documented for all concentrations (Arifin, Nasution and Halimatussakdiah, 2012; Reegan *et al.*, 2015).

The effectiveness of *oviposition deterrence* was evaluated by comparing the number of eggs in the treatment bowls to those in the negative control bowls. The *Effective Repellency* (% ER) and *Oviposition Activity Index* (OAI) were calculated using standard formulas.

$$\% \text{ ER} = \frac{NC - NT}{NC} X\ 100\%$$

$$(\text{OAI}) = \frac{\text{NT-NC}}{\text{NT +NC}}$$

Description:

% ER = Percentage of *effective repellency*
NC = Number of eggs in negative control
NT = Number of eggs in sample
OAI = *Oviposition Activity Index* (Arifin, Nasution and Halimatussakdiah, 2012).

3. RESULTS AND DISCUSSION

3.1 Extraction Results

The methanol extract was evaporated using a rotary evaporator. This resulted in 30.5 g of concentrated methanolic extract of dragon scale fern leaves.

3.2 Phytochemical Screening Results

Phytochemical screening was carried out with the aim of identifying the secondary metabolite compounds contained in the methanol extract of dragon scale fern leaves. The results of the phytochemical screening of the methanol extract showed that the methanol extract of dragon scale fern leaves contained secondary metabolites, namely steroid compounds, saponins, flavonoids and tannins, as shown in Table 1.

Table 1. Phytochemical Test Results of Dragon Scale Fern Leaf Extract (*P. piloselloides* (L.) M. G. Price.

Compound	Reagent	Result
Alkaloids	Dragendorff	-
	Mayer	-
	Wagner	-
Terpenoids	Lieberman Burchard	-
Steroids	Lieberman Burchard	+
Saponins	Aquades	+
phenolic	$FeCl_3$	-
Flavonoids	NaOH	+
Taninns	- $FeCl_3$	+

Infirmation: +: positive; -: negative

The steroid test produces a positive result when the terpenoid test yields a negative outcome. In the steroid test, a color change to green in the solution indicates the presence of steroid compounds. The positive reaction occurs due to condensation or water release, coupled with carboxylation and fusion. The process begins with the release of hydrogen atoms and their electrons, causing shifts in double bonds. This compound then undergoes resonance, acting as an electrophile during carboxylation. Carboxylation triggers

electrophilic addition, followed by the release of hydrogen atoms. Subsequently, the hydrogen atoms and their electrons are released, leading to conjugate extension of the compound. This results in the formation of a red ring, as described in (Nurjannah *et al.*, 2022). The reaction mechanism for the Lieberman-Burchard steroid test is illustrated in Figure 1.

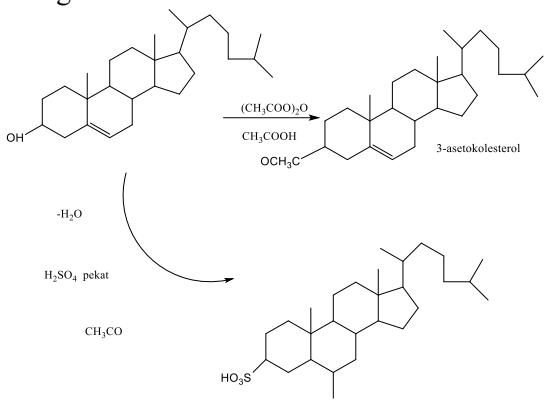

Figure 1. Mechanism of Steroid Test Reaction with Lieberman-Burchard Reagent (Putri Anggraeni, Chatri and Advinda, 2023).

Table 1 showed positive results in the saponin test in methanol extract, as evidenced by the formation of stable foam for 30 minutes, indicating the presence of glycosides with the ability to form foam in water, which are hydrolysed to glucose. The reaction that occurs during the saponin test is shown in Figure 2.

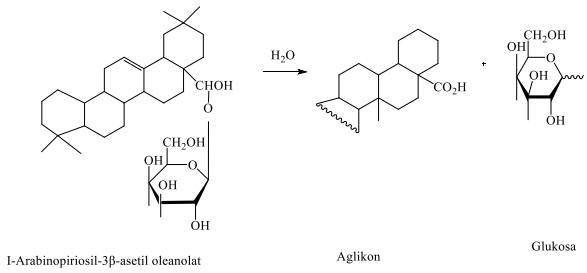

Figure 2. Reaction Mechanism for Testing the Existence of Saponin Compounds (Marlina, Suryati and Suyono, 2005)

The flavonoid test carried out showed positive results, indicated by a change in colour to yellow after testing with 10% NaOH. Crystalline compounds which are derivatives of flavonoid compounds are decomposed by bases in the addition of 10% NaOH into molecules such as acetophenone which is yellow in colour due to the breaking of bonds in the isoprene structure. The reaction mechanism of the flavonoid test with 10% NaOH is shown in Figure 3.

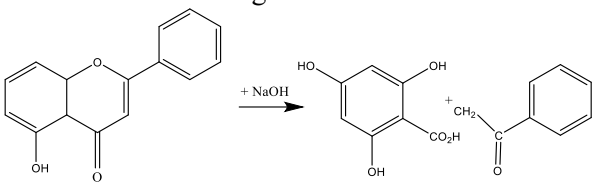

Figure 3. Flavonoid Test Reaction Mechanism with 10% NaOH (Kusnadi and Devi, 2017)

The tannin test carried out showed positive results, characterised by a brownish-green discolouration. This colour change is due to the formation of a complex compound between the Fe metal and the tannin. Complex compounds are formed due to the presence of coordination covalent bonds between metal and non-metalions (Deti Andasari, Hermanto and Wahyuningsih, 2020). The reaction mechanism that occurs in the tannin test is shown in Figure 4.

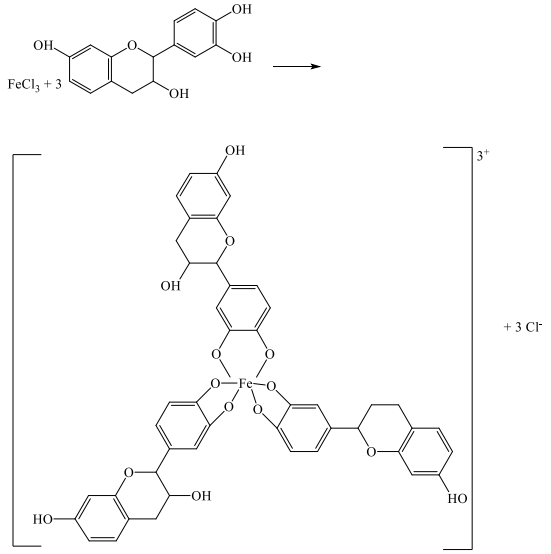

Figure 4. Reaction Mechanism of Tannin Test with $FeCl_3$ (Manongko, Sangi and Momuat, 2020)

3.3 Results of Larvicidal Activity Tests on Culex sp. Larvae

Larvicides aim to eliminate mosquito larvae using chemical compounds. Natural

larvicides offer several benefits, including their ability to break down rapidly under the influence of sunlight, air, moisture, and other environmental factors, thereby minimizing the risk of groundwater and water pollution. Additionally, natural larvicides typically exhibit low toxicity to mammals, making them safer for use around humans. When selecting extracts for use as natural larvicides, it is essential to consider their safety for humans and other organisms, as well as their potential benefits.

A larvicidal test using methanol extract was performed to evaluate the activity of secondary metabolites from the leaves of the dragon scale fern (*P. piloselloides* (L.) M.G. Price) against mosquito larvae. The test assessed larvicidal activity using the Lethal Concentration 50% (LC_{50}) parameter, which represents the concentration of a chemical substance capable of causing the death of 50% of the test organisms. The effectiveness was determined based on the number of larvae killed by the methanol extract of dragon scale fern leaves after 48 hours, as shown in Table 2.

Based on Table 2, above, there are differences in the effect of dragon scale fern leaf extract at each concentration on *Culex sp*. mosquito larvae, where the control treatment with other concentration treatments has different effects. This indicates that there is an influence of chemical compounds that are toxic in the extract so that it can cause death in *Culex sp*. mosquito larvae, so that the concentration that can kill 50% of the *Culex sp*. mosquito population (LC_{50}) can be determined. The number of larvae in Table 2 was entered and analysed in the Bill test table (Table 3) to obtain cumulative data (number of dead accumulated and number of alive accumulated), the data helped to see the overall pattern of number of deaths and survival up to each concentration. Based on these data, the % mortality can be calculated, i.e. the percentage of deaths at each concentration. The curve of % mortality vs. log concentration of the methanol extract can then be plotted in Figure 5, giving a linear regression equation to determine the LC_{50} value.

Table 2. Number of Larvae Killed by Methanol Extract of Dragon Scale Fern Leaves in Larvicidal Activity Test After 48 Hours.

Mosquito Condition	Average Number of Larvae in Methanol Extract Media at Various Concentrations (ppm)							Control (+)	Control (-)
	2000	1000	500	250	125	62.50	31.25		
Life	1	3	6	5	6	7	4	0	10
Dead	9	7	4	5	4	3	6	10	0

Information: - positive control: aquadest + abate; - negative control: aquadest

Table 3. Results of Analysis of Larvicidal Activity of Methanol Extract of Dragon Scale Fern Leaves against Culex sp. Mosquito Larvae using Bill Test Table.

Concentration Log	Death toll	Number of Lives	Accumulated Death Toll	Accumulated Life Count	Ratio	Mortality	LC_{50} (ppm)
					Totally Dead	(%)	
3.301	9	1	38	1	0.97	97.44	
3.000	7	3	29	4	0.88	87.88	
2.699	4	6	22	10	0.69	68.75	
2.398	5	5	18	15	0.55	54.55	143 ppn
2.097	4	6	13	21	0.38	38.24	
1.796	3	7	9	28	0.24	24.32	
1.495	6	4	6	32	0.16	15.79	

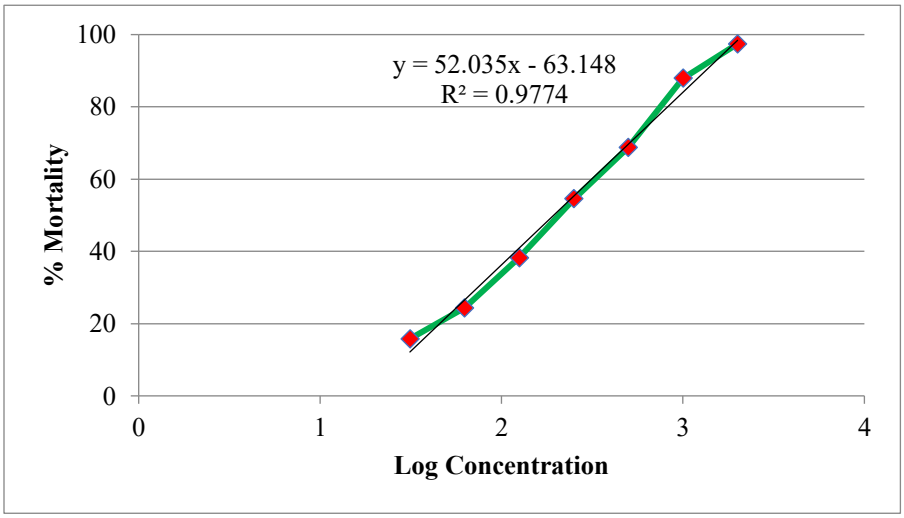

Figure 5. Larvicidal Activity Curve of Dragon Scale Fern Extract (*P. piloselloides* (L.) M. G. Price)

The LC_{50} value was calculated to determine the toxicity of the extract to the test larvae after 72 hours of observation. According to (Sumihe, Runtuwene and Rorong, 2014), the toxicity level of an extract is as follows: if $LC_{50} \leq 30$ mg/L = highly toxic, meaning that a very low concentration is required to kill 50% of the test larvae; if $LC_{50} \leq 1000$ mg/L = toxic, meaning that extracts in this range are also effective as larvicides but require higher concentrations than highly toxic extracts; and if $LC_{50} > 1000$ mg/L = non-toxic, this is considered to be non-toxic to larvae because very high concentrations are not required to achieve 50% mortality, extracts in this category are considered to be less effective as larvicides.

Based on Figure 5. The LC_{50} value was calculated using the linear regression equation obtained from the relationship between the percentage of larval mortality (y) and the log dose (x), namely y = 52.032x - 63.148, where y is the percentage of 50% mortality. This gave an LC_{50} of 143 ppm, which means that at this concentration the extract caused the death of 50% of the larval population within 72 hours. This indicates that the methanol extract of dragon scale fern is toxic and has significant larvicidal activity against *Culex sp*. mosquito larvae.

Figure 5. also shows the correlation coefficient (R^2) = 0.9774 (close to 1) which explains that the relationship between log dose and percentage larval mortality is very strong, which means that this regression model is able to explain about 97.74% of the variation in mortality data observed based on the dose given. The higher the concentration of methanol extract of dragon scale fern leaves, the higher the mortality of *Culex sp*. mosquito larvae, which means the extract is effective as a larvicide.

Bioactive compounds found in plants play a role in killing mosquito larvae; flavonoid compounds work by entering the mosquito's respiratory tract and causing the mosquito's nerves and respiratory muscles to wither so that the mosquito cannot breathe and eventually dies. Saponin compounds work by irritating the lining of the larvae's digestive tract. Saponins also have a bitter effect on the larvae, damaging the waxy layer that protects the insect's outer body, causing the insect to lose a lot of fluid in its body and leading to death. Tannin compounds cause a decrease in the activity of protease enzymes in the

conversion of amino acids. The process of cellular metabolism in the larvae can be disrupted, so the larvae will lack nutrients. In addition, tannins will bind to proteins in the digestive system that the larvae need for growth. If this continues, the larvae will die. The steroid content also affects the larvae, inhibiting the molting process when ingested (Melita, Elsyana and Ulfa, 2022). Steroid compounds are also one of the causes of death in *Culex sp*. mosquito larvae, because steroid compounds are molting inhibitors of mosquito larvae, which cause inhibition of larval development due to failure of metamorphosis of *Culex sp*. mosquito larvae (Ratnawati, Manaf and Sari, 2016).

3.4 Results of Oviposition Deterrent Activity Test of Culex sp. Mosquitoes

The results of the *oviposition deterrent* test of the methanol extract of the leaves of dragon scale fern (*P. piloselloides* (L.) M. G. Price) against *Culex sp*. mosquitoes compared with the activity of abate (positive control) are given in Table 4.4. *Oviposition Deterrent* activity is determined using the % ER parameter to measure the effectiveness of a substance or material in repelling specific insects or organisms. In addition, the OAI is used as a parameter to measure the preference of female insects to lay eggs on a particular extract. Both parameters were determined from the number of *Culex sp*. mosquito eggs at each test concentration. The data are presented in Table 4.

Based on Table 4 above, each concentration of methanol extract has a different effect on the number of eggs laid. At higher concentrations, the number of eggs laid is lower because the effect of the active compounds in the extract is greater, so the activity is stronger, but it will not always be linear. Optimum concentration will have the most significant effect.

The % ER is a metric used to assess a substance's ability, particularly a chemical compound or natural extract, to deter insects from a specific source. This measure evaluates the effectiveness of repellents by calculating the percentage reduction in egg-laying activity due to the extract. Factors influencing % ER include the type of compound, its concentration, volatility, and the insect species involved (sabila, Rahadian and Hidayat, 2013).

OAI is another parameter used to evaluate the preference or avoidance of female insects, such as mosquitoes, for egg-laying sites. The OAI value ranges from -1 to 1, where a value less than 0 indicates a repellent effect, a value greater than 0 indicates an attractant effect, and a value of 0 signifies no significant impact, either as a repellent or attractant.

The percentage values of % ER and OAI of the *oviposition deterrent* activity of methanol extract against the negative control can be seen in Table 5.

Table 5 shows that the methanol extract of the leaves of the dragon scale fern has a strong effect on reducing the number of eggs laid by female *Culex* mosquitoes, with % ER values reaching 72% at a concentration of 2000 ppm.

Table 4. Number of *Culex sp*. Mosquito Eggs in the *Oviposition Deterrent* Activity Test of Dragon Scale Fern Leaf Methanol Extract.

Sample	Number of *Culex sp*. Mosquito Eggs (items)								
	Sample concentration (ppm)							Positive Control	Negative Control
	2000	1000	500	250	125	62.50	31.25		
Methanol extract	7	10	10	14	8	8	12	0	25

Information: - positive control: aquadest + abate; - negative control: aquadest

This percentage indicates that 72% of the female mosquitoes avoided the container

containing the sample extract to lay eggs, which means that the egg-laying inhibitory effect is very significant at high concentrations. Dragon scale fern extract had anti-oviposition effect on female *Culex sp*. mosquitoes at all concentrations (31.25; 62.50; 125; 250; 500; 1000; and 2000 ppm) with OAI values of -0.35; -0.51; -0.51; -0.28; -0.43; -0.43; and -0.56, respectively.

Table 5. Results of Analysis of *Oviposition Deterrent* Activity of Methanol Extract of Dragon Scale Fern Leaves against *Culex sp*. mosquitoes.

Concentration (ppm)	Number of Eggs		ER (%)	OAI
	NC	NT		
2000	25	7	72	-0.56
1000		10	60	-0.43
500		10	60	-0.43
250		14	44	-0.28
125		8	68	-0.51
62.50		8	68	-0.51
31.25		12	52	-0.35

Chakraborty, Burman and Chandra (2022) reported that the chloroform-methanol extract of *Callosobruchus chinensis* seed coat at a concentration of 500 ppm resulted in 79% inhibition of egg laying in *Culex quinquefasciatus* mosquitoes, whereas the results of the larvicidal test against *Culex sp*. mosquitoes in the present study showed 60% inhibition of egg laying at a concentration of 500 ppm. This is because chloroform-methanol extracts often have a higher percent %ER than pure methanol extracts because they can dissolve a wider range of bioactive compounds. Differences in mosquito species can also cause variations in response to certain compounds.

In addition, Chakraborty, Burman and Chandra (2022) reported the OAI value with a result of -0.66 at a concentration of 500 ppm, indicating that the concentration has a repellent effect on *C. quinquefasciatus* mosquitoes. While the calculation of the OAI value at a concentration of 500 ppm in this study is -0.43, which shows that the dragon scale fern extract has a repellent effect on *Culex sp*. This may be caused by the different content of secondary metabolites in the two extracts. The compounds contained in the *C. chinensis* seed coat extract are alkaloids, terpenoids, phenolics, flavonoids, tannins and phytosterols, while the dragon scale fern extract contains only 4 compounds, which is the reason why the OAI value is different even though the concentration is the same.

Flavonoids are believed to act on mosquito eggs by penetrating the eggshell through polygonal pores, leading to structural damage. Similarly, saponins are thought to affect *Culex sp*. mosquito eggs by disrupting the egg wall structure, altering cell wall permeability, and causing fluid loss from the cell, ultimately resulting in dehydration (Madona *et al.*, 2020). Additionally, tannins and steroids are compounds known to actively deter oviposition due to their repellent properties.

The negative control used in the study was aquadest. Based on Table 5 it can be seen that the number of mosquito eggs found in the negative control is higher than the number of eggs found in the methanol extract of dragon scale fern leaves. This is because the methanol extract contains active compounds that make the environment unattractive or uncomfortable for female mosquitoes to lay eggs. In contrast, aquadest, the negative control, did not contain any inhibitory compounds, so female mosquitoes tend to choose aquadest as a safer place to lay their eggs. The extract of dragon scale fern leaves also has a different aroma and taste compared to aquadest. The aroma or taste of the active compounds in dragon scale fern leaves can act as a natural repellent for female

mosquitoes, so they avoid laying eggs in water containing the extract (Abbas *et al.*, 2024).

Based on the results of the study, dragon scale fern extract showed effective larvicidal and *oviposition deterrent* activity against *Culex sp*. This is due to the presence of active compounds such as steroids, saponins, flavonoids and tannins. The combination of the four compounds provides an optimal effect in larvicidal and *oviposition deterrent* activity.

4. CONCLUSIONS

Based on the results of this study, it can be concluded as follows:

1. Secondary metabolite compounds contained in the extract of dragon scale fern (*P. piloselloides* (L.) M. G. Price) are steroid, saponin, flavonoid, and tannin compounds
2. Larvicidal activity of methanol extract of dragon scale fern (*P. piloselloides* (L.) M. G. Price) which is effective against the death of *Culex sp*. larvae obtained an LC_{50} value of 143 ppm
3. Strong *oviposition deterrent* activity of methanol extract of dragon scale fern (*P. piloselloides* (L.) M. G. Price) against *Culex sp*. mosquitoes is found at a concentration of 2000 ppm with an % ER of 72% and at a concentration of 2000 ppm with an OAI value of -0.56.

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