The potential of *Barringtonia asiatica* Biopesticide from Papua to Eradicate Pests in Aquaculture

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ABSTRACT

[*The potential of *Barringtonia asiatica* Biopesticide from Papua to Eradicate Pests in Aquaculture*] *Saponins are compounds derived from plants that are currently widely used in aquaculture for aquatic pest control. In this study, potential saponin components extracted from fish poison tree (Barringtonia asiatica), which is widely dispersed in tropical areas worldwide, were characterized. Saponin properties were obtained from seeds and leaves by extraction and spectrophotometric methods. To test its properties, four different concentrations of saponin extracts, namely 10 ppm, 20 ppm, 30 ppm, and 40 ppm, were applied in four trials containing 20 tilapias each. An unexpected result was obtained and proved that the saponins extracted from the seeds of the fish poison tree proved to be significantly more effective than the saponins extracted from the peel of its fruit. The results also revealed that the lethal dose of saponins reached higher level at a concentration of 40 ppm. It can be concluded that fish poison tree as a poisonous tree plays an important role in ensuring the sustainability of saponin stocks. The use of natural materials such as fish poison tree as a biopesticide has the potential to minimize environmental damage and reduce costs for aquaculture.*

KEYWORDS: *Aquaculture; Barringtonia asiatica; fish poison tree; pests; saponins*

ABSTRAK

Saponin merupakan senyawa berasal dari tanaman yang saat ini banyak digunakan dalam budidaya perikanan, pemanfaatannya banyak digunakan untuk pengendalian hama perairan. Dalam penelitian ini, kami mengkarakterisasi komponen saponin potensial yang diekstraksi dari pohon beracun, yang disebut keben, Barringtonia asiatica, tanaman yang memiliki distribusi luas di daerah tropis di seluruh dunia. Kami memperoleh sifat saponin dari biji dan daun dengan ekstraksi dan metode spektrofotometri. Untuk menguji kemampuan sifat-sifatnya, ekstraksi saponin diterapkan pada empat uji coba berbeda yaitu 10, 20, 30 dan 40 ppm yang masing-masing berisi 20 ekor ikan. Kami memperoleh hasil yang tidak terduga yang membuktikan bahwa saponin yang diekstraksi dari biji keben terbukti berpengaruh signifikan dibandingkan dengan kulit buah. Hasil penelitian juga mengungkapkan bahwa kondisi mematikan saponin mencapai tingkat yang lebih tinggi pada konsentrasi 40 ppm. Kami menyimpulkan, keben sebagai pohon beracun berperan penting untuk menjamin keberlanjutan stok saponin. Penggunaan bahan alami seperti keben sebagai biopestisida berpotensi memberikan dampak yang baik untuk lingkungan dan mengurangi biaya pada usaha budidaya perikanan

KATA KUNCI*: Barringtonia asiatica*; budidaya; hama; keben; saponin

Introduction

Preparations that need to be made for a fish farming business include dewatering and drying of the existing pond, repairing leaks, cleaning residual waste, repairing inlet and outlet, fertilizing, liming, and filling the pond with water. One of the most important stages of pond preparation is drying the pond bottom with the aim of controlling predators, competitors, and some nuisance pests such as wild fish, snakes, frogs, turtles, crabs, and others that hinder and reduce aquaculture production, which is a determining factor for business success (Akbar, 2020). Controlling pests in fish farming areas is a good management practice for food safety and improvement of fish production (Duke *et al*., 2010)

So far, fish farmers generally use synthetic pesticides to control pests. Synthetic pesticides are considered economical, more efficacious in enhancing structural changes, and easy to obtain (Fredricks *et al*., 2021). However, the use of synthetic pesticides will have residual impacts on the environment and health of humans and other living creatures such as wild animals. Synthetic pesticides use hazardous chemicals, such as endosulfan that is contained in pesticides with trademarks Akodan and Thiodan, which are used in the land preparation process *(*Rairakhwada *et al*., 2007). In addition, synthetic pesticides have an impact on cultivated biota. It slows down the growth process, reduces health, and lowers the immune system of the biota (Maqsood *et al*., 2009). Furthermore, synthetic pesticide can cause residual toxicity that spreads in the food chain, causing poisoning for mammals, and it requires preventive measures and operational technical skills in managing it (Yadav & Devi, 2017).

Therefore, it is necessary to find alternative pesticides to save the ecosystem and keep the environment safe. However, in reality, the use of natural pesticides to control pests in aquaculture is still not optimal. Pests in fish ponds can be controlled by using natural ingredients such as saponins. Saponins, which include glycosides, triterpenes and sterols, are secondary metabolites that have the ability to induce hemolysis in fish and cause poisoning in livestock (Harborne, 1987). Saponin compounds can kill wild fish without killing cultured fish, so they are suitable for controlling pests in ponds (Umaru *et al.*, 2018).

These natural compounds are environmentally friendly, easily degraded, and have no negative impact on the environment (Ferdous *et al*., 2018). Imported natural pesticides are difficult to obtain in the eastern part of Indonesia, especially Papua and West Papua, and they are relatively expensive. However, on the other hand, natural pesticides are the right choice because they are environmentally friendly and have multiple effects, namely, eradicating pests, killing invasive species (Mrozik *et al*., 2019), and serving as natural fertilizers after a certain period of time (Ahmad *et al*., 2021). Several local plant species have the potential to control pests in cultivation activities and can be used as pesticides on a large scale because they are biodegradable, food safe, and environment friendly (Ferdous *et al*., 2018).

One material that has active compounds that can be used as natural pesticides is the fruit of fish poison tree (*Barringtonia asiatica*), which can be locally obtained in Papua and has not been used widely. Utilization of this fruit is an appropriate and innovative measure to reduce the use of synthetic pesticides that harm environmental ecosystems. Fish poison tree fruit contains a lot of saponins (Montes De Oca *et al.*, 2017; Umaru, *et al*., 2018; Natawigena *et al*., 2018), terpenes, alkaloids, triterpenoids, phenolics, and tannins (Umaru *et al*., 2019). *Barringtonia asiatica* has several benefits, among others, to treat stomach pain and rheumatism. Its fruit and seeds are used as fish poison. The juice of the fruit is used to treat scabies, while the seeds can also be used as an anthelmintic and the bark is a cure for tuberculosis.

Barringtonia asiatica is a tree that is commonly found along the coast. The fruit of the fish poison tree (B. asiatica) is dispersed by floating at the sea during the rainy season. This plant then thrives and is widely dispersed widely on islands and beaches (Quigley *et al*., 2014). Fish poison tree (*B. asiatica*) is a tropical tree in mangrove forests whose characteristics are that it can grow on sandy beaches, corals and sand plains or river banks, mangrove swamps, and limestone hillsides and is resistant to salt water. *B. asiatica* is widely distributed from East Africa, Pemba Island, Comoro Islands, Madagascar, Seychelles, Mauritius, Chagos Archipelago, India, Sri Lanka, Andaman Islands, Thailand, Cambodia, to Vietnam (Sourav, 2019).

A previous study reported that aqueous crude extract from the seeds of fish poison tree (*B. asiatica*) fruit showed high toxicity and adversely affected the hatchability and lethality of brine shrimp in a test (Mojica & Micor, 2007). In a study, it is also known that methanol extracts and n-hexane, dichloromethane, ethyl acetate, and butanol fractions of the leaves, fruits, seeds, stems, and root bark of *B. asiatica* have antimicrobial activity (Umaru *et al*., 2019: Umaru *et al*., 2020). Furthermore, the results of another study on *B. asiatica* revealed that *B. asiatica* contains saponins which are suspected to function as antifeedant against *Epilachna sp.* larvae (Herlt *et al*., 2002). In addition, Burton *et al.* (2003) discovered ranuncoside, which is oleanane glycoside that has piscicidal activity, in fish poison tree (Rumampuk *et al*., 2010).

Triterpenes that were obtained from the extracts of bark, roots, and flowers of fish poison tree (*B. asiatica*) displayed antimicrobial activity. The extracts obtained by conventional cold-water immersion method using nonpolar, semipolar, and polar solvents (hexane, dichloromethane, chloroform, ethyl acetate, and methanol) exhibited antimicrobial activity against fungal pathogenic strains. A testing on fish poison tree has shown that the plant has antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. The result of the minimum inhibitory concentration testing on hexane extract showed a significant inhibition against *Aspergillus niger* at a dose of 500 g/mL with an inhibition zone of 14.77±0.05 mm. In addition, the dichloromethane crude leaf extract showed higher inhibition against the growth of *Fusarium oxysporium* with an inhibition zone of 15.93 ± 0.05 mm (Umaru *et al*., 2019).

Based on the description of several facts from previous research, it can be said that tropical plant such as fish poison tree has the potential to be used widely as a natural pesticide because it contains saponin compounds. In addition, fish poison tree (*B. asiatica*) exhibits antibacterial, antifungal, and antimicrobial activities (Umaru *et al*., 2020) because it contains secondary metabolites, including saponins and terpenoids (Montes De Oca *et al*., 2017; Umaru *et al*., 2018; Natawigena *et al*., 2018), terpenes, alkaloids, triterpenoids, phenolics, and tannins (Umaru *et al*., 2019). Saponins are triterpene and sterol glycosides, having the ability to induce hemolysis in fish and cause poisoning in livestock (Harborne, 1987). The purpose of this study was to determine the most effective dose of saponins that could be used to control pests in ponds based on the results of phytochemical screening test on seeds and peels of mature and raw fruits of fish poison tree (*B. asiatica*) and based on the result of assessment of saponins as potential biopesticide for aquatic pests.

Materials and Method

The materials used were seeds and peels of raw fruit and mature fruit of fish poison tree (*B. asiatica*), which were obtained from the beach around the campus of the Sorong Marine and Fisheries Polytechnic. Furthermore, this study used tilapias with size ranging from 7 cm to 8 cm that were obtained from the Freshwater Aquaculture Research and Development Installation (IP2BAT) as experimental animals. Before tilapias were used as experimental animals, they were first acclimatized in a controlled vessel for 14 days. Saponins from tea seeds used as control were obtained from commercialized factory processed products.

Phytochemical screening test

Initial screening test is needed to determine the presence of bioactive content in fish poison tree. According to Suhermanto *et al*. (2013) and Harborne (1987), samples need to be extracted using sterile distilled water as a polar solvent for approximately 24 hours. The filtrate obtained was taken as much as 2 mL for the saponin test.

Test for saponin was done by adding alcohol to the sample. A positive test result is indicated by the presence of permanent foam on the surface. Ninety grams of seed powder, raw fruit peel powder, and mature fruit peel powder were taken each and macerated for 24 hours in 500 mL alcohol. The filtrate was then filtered to obtain an alcoholic extract solution. The solution obtained was then evaporated using a rotary evaporator at a temperature of 40ºC until all the solvent evaporated and a concentrated extract was obtained (Harborne, 1987).

Effectiveness test on the administration of *B. Asiatica* extract

The research design used in this study was completely randomized design (CRD). The treatments in this study were different doses with different ranges and control, all of which were repeated 3 times, as follows:

Treatment A (*B. asiatica* seed)	:	administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm
Treatment B (*B. asiatica* raw fruit peel)	:	administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm

Treatment C (*B. asiatica* mature fruit peel)	:	administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm
Control (D)	:	Saponins from tea seeds at a dose of 20 ppm (Shariff *et al.* 2000)

Tilapias were kept in an aquarium with a density of 20 tilapias/aquarium. The research activity was conducted during the day to optimize the performance of the crude extracts of *B. asiatica*. Supporting measures that were conducted were physiological observation of external changes in tilapia and measurements of water qualities, including temperature, pH, and dissolved oxygen (DO), after the treatments were given.

Data analysis

The data obtained from the results of the study were statistically analyzed with one-way analysis of variance (ANOVA) with the help of Minitab Statistical Software version 16 to determine the differences between treatments. Data analysis was then continued with Fisher's extract test. The advantages of *B. asiatica* were described descriptively. The economic value was known by calculating the cost of using saponins in controlling pests in one cycle of whiteleg shrimp cultivation.

Results and Discussion

Fish poison tree (*B. asiatica*) is a plant known with various names, for example, *boton* in the Philippines, *butun*, *putat laut*, *bitung*, and *keben* in Indonesia, and box fruit in India. Fish poison tree is a species of mangrove plant whose habitat is spread on tropical beaches and islands in the Pacific Ocean, India, Zanzibar, Taiwan, Philippines, Fiji, New Caledonia, Solomon Islands, Cook Islands, Wallis and Futuna Islands, and French Polynesia (Quigley *et al*., 2014). This plant likes a wet and humid tropical climate. Its stem growth is episodic and it shows segmentation between the axes with prominent leaf scars. Leaf size varies from small to medium lanceolate leaves 5–20 cm wide in leptocaul species to large ovate-lanceolate leaves 20–40 cm wide (Figure 1).

Figure 1. Leaves, fruit, and seeds of (*B. asiatica)*

Preliminary Test

The formation of long-lasting foams when a plant is extracted or when a plant extract is concentrated is evidence of the presence of saponins (Harborne, 1987). Saponins can be detected in crude extracts based on their ability to induce hemolyis. The results of the preliminary screening of the saponin content in *B. asiatica* can be seen in Table 1.

Table 1. Results of preliminary screening of fish poison tree (*B. asiatica*)

Sample	Solvent	Test result	Description
Seed	HCl	permanent foam	saponin compounds (++)

raw fruit peel			saponin compounds (+)
mature fruit peel			saponin compounds (+)
Seed			saponin compounds (++)
raw fruit peel	Alcohol	permanent foam	saponin compounds (+)
mature fruit peel			saponin compounds (+)

The filtrate that was reacted with concentrated HCl and alcohol gave positive results, indicated by the formation of permanent and dominant foam. Suhermanto *et al.*, (2013) state that if the addition of 3 drops of concentrated HCl into the extract causes the formation of permanent foam, it indicates the content of saponin compounds.

Phytochemical screening test results

The results of phytochemical screening test on fish poison tree indicated the presence of phytochemicals. This can be seen in Table 2.

Table 2. The results of phytochemical screening test on fish poison tree

Extract	Test	Result	Description
Seed	Alkaloid	++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
	Flavonoid	-	No color change
	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change
Raw fruit peel	Alkaloid	++++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
	Flavonoid	++++	Reddish sample solution
	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change
Mature fruit peel	Alkaloid	++++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
	Flavonoid	++++	Reddish sample solution
	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change

The results of the phytochemical screening test showed that all components of fish poison tree contained alkaloids, flavonoids, saponins, and tannins. The yield of fish poison tree seeds of 2.488% contained saponins of 1 g, the peel of raw fruit of fish poison tree with a yield of 0.455% had a saponin content of 0.06 g, and the peel of mature fruit of fish poison tree with a yield of 2.122% had a saponin content of 0.07 g.

Test on the effect of saponins on Tilapia

This test was conducted to determine the effect and effectiveness of saponins from crude extracts of seeds and peels of raw fruit and mature fruit of fish poison tree in controlling pests using tilapia as test animal. The results of the saponin test on tilapia can be seen in Table 3.

Table 3. The results of test on the effect and effectiveness of saponins on tilapia

Sample	**Dosage (ppm)**				**Description**
	10	20	30	40	
Seed	+	+	+	+	Tilapia is dead
raw fruit peel	-	-	-	-	Live tilapia
mature fruit peel	-	-	-	-	Live tilapia

Positive values were shown by the seed extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm, where a dose of 40 ppm was lethal to tilapia. The effect of the administration of saponins derived from fish poison tree seeds on the time of death of tilapia can be seen in Table 4.

Table 4. The effect of the administration of saponin compounds on the time of death of tilapia

Dosage	**Replication**			**Average (minute)**	**Avarage±Stdev**
	1	2	3		
10	118.8	108.8	108.4	112.00	112±5.89
20	92.9	92.9	91.5	92.43	92.43±0.80
30	51.5	48.6	47.0	49.03	49.03±2.28
40	36.5	42.2	39.2	39.30	39.30±2.85

The results of data analysis using one-way analysis of variance (ANOVA) discovered that the administration of saponins at doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm was significantly different between treatments at the 5% level. Saponins displayed other toxic activities, namely, interfering with the regulation of ions and osmotic pressure. This compound can bind oxygen so that oxygen levels in water decrease. This decrease in oxygen levels has a negative impact on fish.

Saponin acts as hemolytic agent. It breaks the bonds of hemoglobin molecules and is responsible for the rupture of the erythrocyte bio-membrane. Phyto-saponins from the seeds of tamarind (*Tamarindus indica*), husks, and sugarcane jaggery are hemotoxic to fish (Das *et al*., 2017). Phyto-saponins are widely used in traditional aquaculture in Asia and Africa for pest control because of their low toxicity to mammals (Clearwater *et al*., 2008).

Saponins are by-product of the manufacturing process of tea seed oil (from *Camellia sinensis*) and are commonly used as organic pesticides in ponds. Tea seeds contain 10-15% saponins, which are effective in controlling pests, but are not lethal to shrimp. Saponins are used as initial and additional pesticides in monoculture and polyculture systems. The dose of saponins commonly used in ponds ranges from 15 to 25 ppm. The higher the salinity is, the lower the dose of saponins applied becomes. Saponins also play a role in stimulating molting in cultured shrimp (Ng'ambi *et al*., 2017), and are used as organic fertilizers that can stimulate algae growth in ponds (Castillo-Ruiz *et al*., 2018).

Saponins contained in organisms generally act as toxic agents to protect the organisms from predators. Invasion of foreign material that is not detected by antigen results in damage to hematopoietic organs so that hemoglobin (Hb) decreases (Suhermanto *et al*., 2011). Changes in hemoglobin content are positively correlated with a decrease in the number of erythrocytes, which is caused by hypochromic microcytic anemia due to infection by saponins. The decrease in hemoglobin concentration is also caused by leukocytosis, which in turn has an impact on the occurrence of erythroblastosis (Harikrishnan *et al*., 2003)

Low hemoglobin causes the metabolic rate of fish to decrease so that the energy produced by fish is low. This condition will have a negative impact on fish. Fish will float on the surface of the water and eventually die (Suhermanto *et al*., 2011). Damage to erythrocytes will also cause disruption of oxygen transport to fish body tissues, thereby inhibiting metabolic processes (Suhermanto *et al*., 2013).

Saponins are harmful to fish, but at certain doses, they have a positive impact on biota. George Francis et al. (2002) revealed that saponins from quillaja that were mixed in feed at a dose of 150 mg per one kilogram of feed could increase the growth of common carp (*C. carpio*) by 372%, while the control increased the growth of common carp by 327%, and treatment of 300 mg per one kilogram of feed increased the growth of common carp only by 325%. George Francis et al. (2005) states that saponins derived from quillaja that are then mixed in feed can increase the growth, metabolism, and reproduction of tilapia. Quillaja mixed in feed does not affect the egg production of tilapia, but instead it can increase its metabolism and growth (George Francis *et al*., 2001).

Research conducted on the peel of raw fruit and mature fruit of fish poison tree indicated that saponins did not have a lethal effect on fish. Administering saponins extracted from fish poison tree to tilapia is suspected to be able to improve the immune response in tilapia. Administration of low and high doses of saponins will be tolerogenic, while the optimum dose can increase the immune response. Doses that are too high can interfere with fish's immune system and will cause damage to organs and cells with the mechanism being unable to tolerate conditions beyond its capabilities.

Foreign substance that enters the body of a biota will be identified in such a way that it is recognized, then it will be processed and sent to the cell-mediated antibody-forming system. Lymphocytes are cells that specifically recognize and respond to external antigens and then act as mediators between cell-mediated immunity and humoral immunity. B lymphocytes are cells with the ability to produce antibodies, recognize extracellular antigens, and differentiate between antibodies in plasma cells. T cells are intermediate immune cells that recognize intracellular antigens and function to destroy microbes or infected cells (Suhermanto *et al*., 2011)

Water quality

The results of measurements of water temperature in the aquariums showed a temperature range between 29ºC–31ºC. Temperature greatly affects the rate of reaction of saponins. The higher the water temperature, the faster the saponins will kill pests. The measurements of the pH of the water during the study obtained a pH value between 7.3 - 7.5, while the measurement of dissolved oxygen in the aquariums showed the results ranging from 6.9 mg/L to 7.3 mg/L.

The advantages of using fish poison tree in aquaculture

Saponins as natural compounds are obtained from tropical plants (Quigley *et al*. 2014) and are used in shrimp culture at the land preparation stage to eradicate pests such as wild fish that become predators, competitors, and nuisances in ponds. Commercial saponin products that are widely used are usually made from the seeds of tea (*Camellia Oleifeira*). However, tea seeds still have to be imported and are expensive in Papua. Fish poison tree as an alternative source of saponins has proven to be effective and can be used to support environmentally friendly and sustainable aquaculture, as can be seen in Table 5.

Table 5. Comparison of saponin applications in land preparation

Ingredients	**Dosage (ppm)**	**Price/kg (IDR)**	**Description**
Seed of tea (*C. Oleifeira*)	15-20	35,000	Impor
Seed of Keben (*B. asiatica*)	10-15	0	Local raw materials

Saponins from the seeds of fish poison tree (*B. asiatica*), referring to the results of the study presented above, have the potential to function as invasive fish control. However, saponins in general are also useful as antimicrobial agent, immunostimulant, and surface tension reducer (Francis *et al*., 2005)

The economic value of the use of saponins derived from fish poison trees for whiteleg shrimp farmers in Sorong, West Papua, can be identified by comparing the use of saponins in the cultivation of whiteleg shrimp. The saponins needed in pond preparation for aquaculture are around 50 kilograms per production cycle, with a price of IDR 35,000 per one kilogram of saponins. Thus, it can at least save production costs of around IDR 1,750,000 for each plot with an area of about 1,700 m^2 and a pond depth of 1-1.5 m.

Another advantage of the use of saponins derived from fish poison tree as biopesticides is that these saponins are local raw materials so they are easy to obtain and low-cost, thus reducing operational costs, and it supports organic farming practices (Ahmed *et al*., 2020) that are safe for cultivated biota (shrimp). In addition, saponins do not pollute waters and biota that are produced from aquaculture that uses saponins as pest control is safe for consumption and good for consumer health.

Conclusion

The results of the phytochemical screening test revealed the presence of secondary metabolites in fish poison tree (*B. asiatica*), including saponins, alkaloids, flavonoids, and tannins, with the highest concentration of bioactive saponins found in seeds. Administration of crude extract of fish poison tree (*B. asiatica*) seeds to test animals showed a positive impact at doses starting at 10 ppm. The higher the dose of saponin, the faster the saponin will eradicate pests. Saponins from fish poison tree seeds have potential benefits as invasive fish repellents. The use of saponins obtained from the seeds of fish poison tree (*B. asiatica*) has economic value because it is from local materials, is environmentally friendly, and is safe for human health.

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The potential of *Barringtonia asiatica* Biopesticide from Papua to Eradicate Pests in Aquaculture

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ABSTRACT

Saponins are compounds derived from plants that are currently widely used in aquaculture for aquatic pest control. In this study, potential saponin components extracted from fish poison tree (Barringtonia asiatica), which is widely dispersed in tropical areas worldwide, were characterized. Saponin properties were obtained from seeds and leaves by extraction and spectrophotometric methods. To test its properties, four different concentrations of saponin extracts, namely 10 ppm, 20 ppm, 30 ppm, and 40 ppm, were applied in four trials containing 20 tilapias each. An unexpected result was obtained and proved that the saponins extracted from the seeds of the fish poison tree proved to be significantly more effective than the saponins extracted from the peel of its fruit to eradicate pest in pond. The results also revealed that the lethal dose of saponins reached higher level at a concentration of 40 ppm. It can be concluded that fish poison tree as a poisonous tree plays an important role in ensuring the sustainability of saponin stocks. The use of natural materials such as fish poison tree as a biopesticide has the potential to minimize environmental damage and reduce costs for aquaculture.

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KEYWORDS: *Aquaculture; Barringtonia asiatica; fish poison tree; pests; saponins*

ABSTRAK

Saponin merupakan senyawa berasal dari tanaman yang saat ini banyak digunakan dalam budidaya perikanan, pemanfaatannya banyak digunakan untuk pengendalian hama perairan. Dalam penelitian ini, kami mengkarakterisasi komponen saponin potensial yang diekstraksi dari pohon beracun, yang disebut keben, Barringtonia asiatica, tanaman yang memiliki distribusi luas di daerah tropis di seluruh dunia. Kami memperoleh sifat saponin dari biji dan daun dengan ekstraksi dan metode spektrofotometri. Untuk menguji kemampuan sifat-sifatnya, ekstraksi saponin diterapkan pada empat uji coba berbeda yaitu 10, 20, 30 dan 40 ppm yang masing-masing diisi 20 ekor ikan. Kami memperoleh hasil yang tidak terduga yang membuktikan bahwa saponin yang diekstraksi dari biji keben terbukti berpengaruh signifikan dalam memberantas hama di kolam dibandingkan dengan kulit buah. Hasil penelitian juga mengungkapkan bahwa kondisi mematikan saponin mencapai tingkat yang lebih tinggi pada konsentrasi 40 ppm. Kami menyimpulkan, keben sebagai pohon beracun berperan penting untuk menjamin keberlanjutan stok saponin. Penggunaan bahan alami seperti keben sebagai biopestisida berpotensi memberikan dampak yang baik untuk lingkungan dan mengurangi biaya pada usaha budidaya perikanan

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KATA KUNCI*: Barringtonia asiatica*; budidaya; hama; keben; saponin

Introduction

Preparations that need to be made for a fish farming business include ~~dewatering~~ and drying of the existing pond, repairing leaks, cleaning residual waste, repairing inlet and outlet, fertilizing, liming, and filling the pond with water. One of the most important stages of pond preparation is drying the pond bottom with the aim of controlling predators, competitors, and some nuisance pests such as wild fish, snakes, frogs, turtles, crabs, and others that hinder and reduce aquaculture production, which is a determining factor for business success (Akbar, 2020). Controlling pests in fish farming areas is a good management practice for food safety and improvement of fish production (Duke et al., 2010)

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So far, fish farmers generally use synthetic pesticides to control pests. Synthetic pesticides are considered economical, more efficacious in enhancing structural changes, and easy to obtain (Fredricks, Hubert, Amberg, Cupp, & Dawson, 2021). However, the use of synthetic pesticides will have residual impacts on the environment and health of humans and other living creatures such as wild animals. Synthetic pesticides use hazardous chemicals, such as endosulfan that is contained in pesticides with trademarks Akodan and Thiodan, which are used in the land preparation process *(*Rairakhwada et al., 2007). In addition, synthetic pesticides have an impact on cultivated biota. It slows down the growth process, reduces health, and lowers the immune system of the biota (Maqsood, Samoon, & Singh, 2009). Furthermore, synthetic pesticide can cause residual toxicity that spreads in the food chain, causing poisoning for mammals, and it requires preventive measures and operational technical skills in managing it (Yadav & Devi, 2017).

Therefore, it is necessary to find alternative pesticides to save the ecosystem and keep the environment safe. However, in reality, the use of natural pesticides to control pests in aquaculture is still not optimal. Pests in fish ponds can be controlled by using natural ingredients such as saponins. Saponins, which include glycosides, triterpenes and sterols, are secondary metabolites that have the ability to induce hemolysis in fish and cause poisoning in livestock (Harborne, 1987). Saponin compounds can kill wild fish without killing cultured fish, so they are suitable for controlling pests in ponds (Umaru et al., 2018).

These natural compounds are environmentally friendly, easily degraded, and have no negative impact on the environment (Ferdous, Rana, & Habib, 2018). Imported natural pesticides are difficult to obtain in the eastern part of Indonesia, especially Papua and West Papua, and they are relatively expensive. However, on the other hand, natural pesticides are the right choice because they are environmentally friendly and have multiple effects, namely, eradicating pests, killing invasive species (Mrozik et al., 2019), and serving as natural fertilizers after a certain period of time (Ahmad, Sheikh Abdullah, Hasan, Othman, & Ismail, 2021). Several local plant species have the potential to control pests in cultivation activities and can be used as pesticides on a large scale because they are biodegradable, food safe, and environment friendly (Ferdous et al., 2018).

One material that has active compounds that can be used as natural pesticides is the fruit of fish poison tree (*Barringtonia asiatica*), which can be locally obtained in Papua and has not been used widely. Utilization of this fruit is an appropriate and innovative measure to reduce the use of synthetic pesticides that harm environmental ecosystems. Fish poison tree fruit contains ~~a lot of~~ saponins (Montes De Oca et al., 2017; Umaru, et al., 2018; Natawigena et al., 2018), terpenes, alkaloids, triterpenoids, phenolics, and tannins (Umaru et al., 2019). *Barringtonia asiatica* has several benefits, among others, to treat stomach pain and rheumatism. Its fruit and seeds are used as fish poison. The juice of the fruit is used to treat scabies, while the seeds can also be used as an anthelmintic and the bark is a cure for tuberculosis (Ragasa et al., 2014).

Barringtonia asiatica is a tree that is commonly found along the coast. The fruit of the fish poison tree (B. asiatica) is dispersed by floating at the sea during the rainy season. This plant then thrives and is widely dispersed widely on islands and beaches (Quigley, Gainey, & Dinsdale, 2014). Fish poison tree (*B. asiatica*) is a tropical tree in mangrove forests whose characteristics are that it can grow on sandy beaches, corals and sand plains or river banks, mangrove swamps, and limestone hillsides and is resistant to salt water. *B. asiatica* is widely distributed from East Africa, Pemba Island, Comoro Islands, Madagascar, Seychelles, Mauritius, Chagos Archipelago, India, Sri Lanka, Andaman Islands, Thailand, Cambodia, to Vietnam (Sourav, 2019) and Indonesia (Rumampuk et al., 2003).

A previous study reported that aqueous crude extract from the seeds of fish poison tree (*B. asiatica*) fruit showed high toxicity and adversely affected the hatchability and lethality of brine shrimp in a test (Mojica & Micor, 2007). In a study, it is also known that methanol extracts and n-hexane, dichloromethane, ethyl acetate, and butanol fractions of the leaves, fruits, seeds, stems, and root bark of *B. asiatica* have antimicrobial activity (Umaru et al., 2019: Umaru et al., 2020). Furthermore, the results of another study on *B. asiatica* revealed that *B. asiatica* contains saponins which are suspected to function as antifeedant against *Epilachna sp.* larvae (Herlt et al., 2002). In addition, Burton et al. (2003) discovered ranuncoside, which is oleanane glycoside that has piscicidal activity, in fish poison tree (Rumampuk et al., 2003).

Triterpenes that were obtained from the extracts of bark, roots, and flowers of fish poison tree (*B. asiatica*) displayed antimicrobial activity. The extracts obtained by conventional cold-water immersion method using nonpolar, semipolar, and polar solvents (hexane, dichloromethane, chloroform, ethyl acetate, and methanol) exhibited antimicrobial activity against fungal pathogenic strains. A testing on fish poison tree has shown that the plant has antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. The result of the minimum inhibitory concentration testing on hexane extract showed a significant inhibition against *Aspergillus niger* at a dose of 500 g/mL with an inhibition zone of 14.77±0.05 mm. In addition, the dichloromethane crude leaf extract showed higher inhibition against the growth of *Fusarium oxysporium* with an inhibition zone of 15.93 ± 0.05 mm (Umaru et al., 2019).

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Based on the description of several facts from previous research, it can be said that tropical plant such as fish poison tree has the potential to be used widely as a natural pesticide because it contains saponin compounds. In addition, fish poison tree (*B. asiatica*) exhibits antibacterial, antifungal, and antimicrobial activities (Umaru et al., 2020) because it contains secondary metabolites, including saponins and terpenoids (Montes De Oca et al., 2017; Umaru et al., 2018; Natawigena et al., 2018), terpenes, alkaloids, triterpenoids, phenolics, and tannins (Umaru et al., 2019). Saponins are triterpene and sterol glycosides, having the ability to induce hemolysis in fish and cause poisoning in livestock (Harborne, 1987). The purpose of this study was to determine the most effective dose of saponins that could be used to control pests in ponds based on the results of phytochemical screening test on seeds and peels of mature and raw fruits of fish poison tree (*B. asiatica*) and based on the result of assessment of saponins as potential biopesticide for aquatic pests.

Materials and Method

The materials used were seeds and peels of raw fruit and mature fruit of fish poison tree (*B. asiatica*), which were obtained from the beach around the campus of the Sorong Marine and Fisheries Polytechnic. Furthermore, this study used tilapias with size ranging from 7 cm to 8 cm that were obtained from the Freshwater Aquaculture Research and Development Installation (IP2BAT) as experimental animals. Before tilapias were used as experimental animals, they were first acclimatized in a controlled vessel for 14 days. Saponins from tea seeds used as control were obtained from commercialized factory processed products.

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Phytochemical screening test

Initial screening test is needed to determine the presence of bioactive content in fish poison tree. According to Suhermanto et al. (2013) and Harborne (1987), samples need to be extracted using sterile distilled water as a polar solvent for approximately 24 hours. The filtrate obtained was taken as much as 2 mL for the saponin test.

Test for saponin was done by adding alcohol to the sample. A positive test result is indicated by the presence of permanent foam on the surface. Ninety grams of seed powder, raw fruit peel powder, and mature fruit peel powder were taken each and macerated for 24 hours in 500 mL alcohol. The filtrate was then filtered to obtain an alcoholic extract solution. The solution obtained was then evaporated using a rotary evaporator at a temperature of 40ºC until all the solvent evaporated and a concentrated extract was obtained (Harborne, 1987).

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Alcohol PA 99.9%

Effectiveness test on the administration of *B. Asiatica* extract

The research design used in this study was completely randomized design (CRD). The treatments in this study were different doses with different ranges and control, all of which were repeated 3 times, as follows:

Treatment A (*B. asiatica* seed)	:	administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm
Treatment B (*B. asiatica* raw fruit peel)	:	administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm

Treatment C (*B. asiatica* mature fruit peel) : administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm

Control (D) : Saponins from tea seeds at a dose of 20 ppm (Shariff *et al.* 2000)

Tilapias were kept in an aquarium with a density of 20 tilapias/L~~aquarium~~. The research activity was conducted during the day to optimize the performance of the crude extracts of *B. asiatica*. Supporting measures that were conducted were physiological observation of external changes in tilapia and measurements of water qualities, including temperature, pH, and dissolved oxygen (DO), after the treatments were given.

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Data analysis

The data obtained from the results of the study were statistically analyzed with one-way analysis of variance (ANOVA) with the help of Minitab Statistical Software version 16 to determine the differences between treatments. Data analysis was then continued with Fisher's extract test. The advantages of *B. asiatica* were described descriptively. The economic value was known by calculating the cost of using saponins in controlling pests in one cycle of whiteleg shrimp cultivation.

Results and Discussion

Fish poison tree (*B. asiatica*) is a plant known with various names, for example, *boton* in the Philippines, *butun*, *putat laut*, *bitung*, and *keben* in Indonesia, and box fruit in India. Fish poison tree is a species of mangrove plant whose habitat is spread on tropical beaches and islands in the Pacific Ocean, India, Zanzibar, Taiwan, Philippines, Fiji, New Caledonia, Solomon Islands, Cook Islands, Wallis and Futuna Islands, and French Polynesia (Quigley et al., 2014). This plant likes a wet and humid tropical climate. Its stem growth is episodic and it shows segmentation between the axes with prominent leaf scars. Leaf size varies from small to medium lanceolate leaves 5–20 cm wide in leptocaul species to large ovate-lanceolate leaves 20–40 cm wide (Figure 1).

Figure 1. Leaves, fruit, and seeds of (*B. asiatica)*

Preliminary Test

The formation of long-lasting foams when a plant is extracted or when a plant extract is concentrated is evidence of the presence of saponins (Harborne, 1987). Saponins can be detected in crude extracts based on their ability to induce hemolyis. The results of the preliminary screening of the saponin content in *B. asiatica* can be seen in Table 1.

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*2 mL sample, 0.1 mL solvent

Table 1. Results of preliminary screening of fish poison tree (*B. asiatica*)

Sample	Solvent	Test result	Description*
Seed	HCl	permanent foam	saponin compounds (++)

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Metode mengacu pada Suhermanto dan Harborne

raw fruit peel			saponin compounds (+)
mature fruit peel			saponin compounds (+)
Seed			saponin compounds (++)
raw fruit peel	Alcohol	permanent foam	saponin compounds (+)
mature fruit peel			saponin compounds (+)

*2 mL sample, 0.1 mL solvent

The filtrate that was reacted with concentrated HCl and alcohol gave positive results, indicated by the formation of permanent and dominant foam. Suhermanto et al., (2013) state that if the addition of 3 drops of concentrated HCl into the extract causes the formation of permanent foam, it indicates the content of saponin compounds.

Phytochemical screening test results

The results of phytochemical screening test on fish poison tree indicated the presence of phytochemicals. This can be seen in Table 2.

Table 2. The results of phytochemical screening test on fish poison tree

Extract	Test	Result	Description
	Alkaloid	++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
	Flavonoid	-	No color change
Seed	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change
	Alkaloid	++++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
Raw fruit peel	Flavonoid	++++	Reddish sample solution
	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change
	Alkaloid	++++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
Mature fruit peel	Flavonoid	++++	Reddish sample solution
	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change

The results of the phytochemical screening test showed that all components of fish poison tree contained alkaloids, flavonoids, saponins, and tannins. The yield of fish poison tree seeds of 2.488% contained saponins of 1 g, the peel of raw fruit of fish poison tree with a yield of 0.455% had a saponin content of 0.06 g, and the peel of mature fruit of fish poison tree with a yield of 2.122% had a saponin content of 0.07 g.

Test on the effect of saponins on Tilapia

This test was conducted to determine the effect and effectiveness of saponins from crude extracts of seeds and peels of raw fruit and mature fruit of fish poison tree in controlling pests using tilapia as test animal. The results of the saponin test on tilapia can be seen in Table 3.

Table 3. The results of test on the effect and effectiveness of saponins on tilapia

Sample	Dosage (ppm)				Description
	10	20	30	40	
Seed	+	+	+	+	Tilapia is dead
raw fruit peel	-	-	-	-	Live tilapia
mature fruit peel	-	-	-	-	Live tilapia

Positive values were shown by the seed extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm, where a dose of 40 ppm was lethal to tilapia. The effect of the administration of saponins derived from fish poison tree seeds on the time of death of tilapia can be seen in Table 4.

Table 4. The effect of the administration of saponin compounds on the time of death of tilapia

Dosage	Replication			Average (minute)	Avarage±Stdev
	1	2	3		
10	118.8	108.8	108.4	112.00	112±5.89
20	92.9	92.9	91.5	92.43	92.43±0.80
30	51.5	48.6	47.0	49.03	49.03±2.28
40	36.5	42.2	39.2	39.30	39.30±2.85

The results of data analysis using one-way analysis of variance (ANOVA) discovered that the administration of saponins at doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm was significantly different between treatments at the 5% level. Saponins displayed other toxic activities, namely, interfering with the regulation of ions and osmotic pressure. This compound can bind oxygen so that oxygen levels in water decrease (Das et al., 2017). This decrease in oxygen levels has a negative impact on fish.

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Saponin acts as hemolytic agent. It breaks the bonds of hemoglobin molecules and is responsible for the rupture of the erythrocyte bio-membrane. Phyto-saponins from the seeds of tamarind (*Tamarindus indica*), husks, and sugarcane jaggery are hemotoxic to fish (Das et al., 2017). Phyto-saponins are widely used in traditional aquaculture in Asia and Africa for pest control because of their low toxicity to mammals (Clearwater et al., 2008).

Saponins are by-product of the manufacturing process of tea seed oil (from *Camellia sinensis*) and are commonly used as organic pesticides in ponds. Tea seeds contain 10-15% saponins, which are effective in controlling pests, but are not lethal to shrimp. Saponins are used as initial and additional pesticides in monoculture and polyculture systems. The dose of saponins commonly used in ponds ranges from 15 to 25 ppm. The higher the salinity is, the lower the dose of saponins applied becomes. Saponins also play a role in stimulating molting in cultured shrimp (Ng'ambi et al., 2017), and are used as organic fertilizers that can stimulate algae growth in ponds (Castillo-Ruiz et al., 2018).

Saponins contained in organisms generally act as toxic agents to protect the organisms from predators. Invasion of foreign material that is not detected by antigen results in damage to hematopoietic organs so that hemoglobin (Hb) decreases (Suhermanto et al., 2011). Changes in hemoglobin content are positively correlated with a decrease in the number of erythrocytes, which is caused by hypochromic microcytic anemia due to infection by saponins. The decrease in hemoglobin concentration is also caused by leukocytosis, which in turn has an impact on the occurrence of erythroblastosis (Harikrishnan et al., 2003)

Low hemoglobin causes the metabolic rate of fish to decrease so that the energy produced by fish is low. This condition will have a negative impact on fish. Fish will float on the surface of the water and eventually die (Suhermanto et al., 2011). Damage to erythrocytes will also cause disruption of oxygen transport to fish body tissues, thereby inhibiting metabolic processes (Suhermanto et al., 2013).

Saponins are harmful to fish, but at certain doses, they have a positive impact on biota. (George Francis, Makkar, & Becker, 2002) revealed that saponins from quillaja that were mixed in feed at a dose of 150 mg per one kilogram of feed could increase the growth of common carp (*C. carpio*) by 372%, while the control increased the growth of common carp by 327%, and treatment of 300 mg per one kilogram of feed increased the growth of common carp only by 325%. (George Francis, Makkar, & Becker, 2005) states that saponins derived from quillaja that are then mixed in feed can increase the growth, metabolism, and reproduction of tilapia. Quillaja mixed in feed does not affect the egg production of tilapia, but instead it can increase its metabolism and growth was 245% over the initial body mass, higher than that of the control group 188% (George Francis et al., 2001).

Research conducted on the peel of raw fruit and mature fruit of fish poison tree indicated that saponins did not have a lethal effect on fish. Administering saponins extracted from fish poison tree to tilapia is suspected to be able to improve the immune response in tilapia. Administration of low and high doses of saponins will be tolerogenic, while the optimum dose can increase the immune response. Doses that are too high can interfere with fish's immune system and will cause damage to organs and cells with the mechanism being unable to tolerate conditions beyond its capabilities.

Foreign substance that enters the body of a biota will be identified in such a way that it is recognized, then it will be processed and sent to the cell-mediated antibody-forming system. Lymphocytes are cells that specifically recognize and respond to external antigens and then act as mediators between cell-mediated immunity and humoral immunity. B lymphocytes are cells with the ability to produce antibodies, recognize extracellular antigens, and differentiate between antibodies in plasma cells. T cells are intermediate immune cells that recognize intracellular antigens and function to destroy microbes or infected cells (Suhermanto et al., 2011)

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Water quality

The results of measurements of water temperature in the aquariums showed a temperature range between 29ºC–31ºC. Temperature greatly affects the rate of reaction of saponins. The higher the water temperature, the faster the saponins will kill pests. The measurements of the pH of the water during the study obtained a pH value between 7.3 - 7.5, while the measurement of dissolved oxygen in the aquariums showed the results ranging from 6.9 mg/L to 7.3 mg/L. The parameters above are still within the optimal range for tilapia maintenance based on SNI 7550:2009.

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The advantages of using fish poison tree in aquaculture

Saponins as natural compounds are obtained from tropical plants (Quigley et al., 2014) and are used in shrimp culture at the land preparation stage to eradicate pests such as wild fish that become predators, competitors, and nuisances in ponds. Commercial saponin products that are widely used are usually made from the seeds of tea (*Camellia Oleifeira*). However, tea seeds still have to be imported and are expensive in Papua. Fish poison tree as an alternative source of saponins has proven to be effective and can be used to support environmentally friendly and sustainable aquaculture, as can be seen in Table 5.

Table 5. Comparison of saponin applications in land preparation

Ingredients	Dosage (ppm)	Price/kg (IDR)	Description
Seed of tea (*C. Oleifeira*)	15-20	35,000	Impor
Seed of Keben (*B. asiatica*)	10-15	0	Local raw materials

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Data merupakan hasil penelitian. Harga merupakan harga pasar

Saponins from the seeds of fish poison tree (*B. asiatica*), referring to the results of the study presented above, have the potential to function as invasive fish control. However, saponins in general are also useful as antimicrobial agent, immunostimulant, and surface tension reducer (Francis et al., 2005)

The economic value of the use of saponins derived from fish poison trees for whiteleg shrimp farmers in Sorong, West Papua, can be identified by comparing the use of saponins in the cultivation of whiteleg shrimp. The saponins needed in pond preparation for aquaculture are around 50 kilograms per production cycle, with a price of IDR 35,000 per one kilogram of saponins. Thus, it can at least save production costs of around IDR 1,750,000 for each plot with an area of about 1,700 m^2 and a pond depth of 1-1.5 m.

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Another advantage of the use of saponins derived from fish poison tree as biopesticides is that these saponins are local raw materials so they are easy to obtain and low-cost, thus reducing operational costs, and it supports organic farming practices (Ahmed et al., 2020) that are safe for cultivated biota (shrimp). In addition, saponins do not pollute waters and biota that are produced from aquaculture that uses saponins as pest control is safe for consumption and good for consumer health.

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Conclusion

The results of the phytochemical screening test revealed the presence of secondary metabolites in fish poison tree (*B. asiatica*), including saponins, alkaloids, flavonoids, and tannins, with the highest concentration of bioactive saponins found in seeds.

Administration of crude extract of fish poison tree (*B. asiatica*) seeds to test animals showed a positive impact at doses starting at 10 ppm. The higher the dose of saponin, the faster the saponin will eradicate pests. Saponins from fish poison tree seeds have potential benefits as invasive fish repellents. The use of saponins obtained from the seeds of fish poison tree (*B. asiatica*) has economic value because it is from local materials, is environmentally friendly, and is safe for human health.

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The potential of *Barringtonia asiatica* Biopesticide from Papua to Eradicate Pests in Aquaculture

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ABSTRACT

Saponins are compounds derived from plants that are currently widely used in aquaculture for aquatic pest control. In this study, potential saponin components extracted from fish poison tree (Barringtonia asiatica), which is widely dispersed in tropical areas worldwide, were characterized. Saponin properties were obtained from seeds and leaves by extraction and spectrophotometric methods. To test its properties, four different concentrations of saponin extracts, namely 10 ppm, 20 ppm, 30 ppm, and 40 ppm, were applied in four trials containing 20 tilapias each. An unexpected result was obtained and proved that the saponins extracted from the seeds of the fish poison tree proved to be significantly more effective than the saponins extracted from the peel of its fruit to eradicate pest in pond. The results also revealed that the lethal dose of saponins reached higher level at a concentration of 40 ppm. It can be concluded that fish poison tree as a poisonous tree plays an important role in ensuring the sustainability of saponin stocks. The use of natural materials such as fish poison tree as a biopesticide has the potential to minimize environmental damage and reduce costs for aquaculture.
Keywords: Aquaculture; Barringtonia asiatica; fish poison tree; pests; saponins

ABSTRAK

Saponin merupakan senyawa berasal dari tanaman yang saat ini banyak digunakan dalam budidaya perikanan, pemanfaatannya banyak digunakan untuk pengendalian hama perairan. Dalam penelitian ini, kami mengkarakterisasi komponen saponin potensial yang diekstraksi dari pohon beracun, yang disebut keben, Barringtonia asiatica, tanaman yang memiliki distribusi luas di daerah tropis di seluruh dunia. Kami memperoleh sifat saponin dari biji dan daun dengan ekstraksi dan metode spektrofotometri. Untuk menguji kemampuan sifat-sifatnya, ekstraksi saponin diterapkan pada empat uji coba berbeda yaitu 10, 20, 30 dan 40 ppm yang masing-masing diisi 20 ekor ikan. Kami memperoleh hasil yang tidak terduga yang membuktikan bahwa saponin yang diekstraksi dari biji keben terbukti berpengaruh signifikan dalam memberantas hama di kolam dibandingkan dengan kulit buah. Hasil penelitian juga mengungkapkan bahwa kondisi mematikan saponin mencapai tingkat yang lebih tinggi pada konsentrasi 40 ppm. Kami menyimpulkan, keben sebagai pohon beracun berperan penting untuk menjamin keberlanjutan stok saponin. Penggunaan bahan alami seperti keben sebagai biopestisida berpotensi memberikan dampak yang baik untuk lingkungan dan mengurangi biaya pada usaha budidaya perikanan

Kata Kunci: Barringtonia asiatica; budidaya; hama; keben; saponi

INTRODUCTION

Preparations that need to be made for a fish farming business include and drying of the existing pond, repairing leaks, cleaning residual waste, repairing inlet and outlet, fertilizing, liming, and filling the pond with water. One of the most important stages of pond preparation is drying the pond bottom with the aim of controlling predators, competitors, and some nuisance pests such as wild fish, snakes, frogs, turtles, crabs, and others that hinder and reduce aquaculture production, which is a determining factor for business success (Akbar, 2020). Controlling pests in fish farming areas is a good management practice for food safety and improvement of fish production (Duke et al., 2010)

So far, fish farmers generally use synthetic pesticides to control pests. Synthetic pesticides are considered economical, more efficacious in enhancing structural changes, and easy to obtain (Fredricks, Hubert, Amberg, Cupp, & Dawson, 2021). However, the use of synthetic pesticides will have residual impacts on the environment and health of humans and other living creatures such as wild animals. Synthetic pesticides use hazardous chemicals, such as endosulfan that is contained in pesticides with trademarks Akodan and Thiodan, which are used in the land preparation process *(*Rairakhwada et al., 2007). In addition, synthetic pesticides have an impact on cultivated biota. It slows down the growth process, reduces health, and lowers the immune system of the biota (Maqsood, Samoon, & Singh, 2009). Furthermore, synthetic pesticide can cause residual toxicity that spreads in the food chain, causing poisoning for mammals, and it requires preventive measures and operational technical skills in managing it (Yadav & Devi, 2017).

Therefore, it is necessary to find alternative pesticides to save the ecosystem and keep the environment safe. However, in reality, the use of natural pesticides to control pests in aquaculture is still not optimal. Pests in fish ponds can be controlled by using natural ingredients such as saponins. Saponins, which include glycosides, triterpenes and sterols, are secondary metabolites that have the ability to induce hemolysis in fish and cause poisoning in livestock (Harborne, 1987). Saponin compounds can kill wild fish without killing cultured fish, so they are suitable for controlling pests in ponds (Umaru et al., 2018).

These natural compounds are environmentally friendly, easily degraded, and have no negative impact on the environment (Ferdous, Rana, & Habib, 2018). Imported natural pesticides are difficult to obtain in the eastern part of Indonesia, especially Papua and West Papua, and they are relatively expensive. However, on the other hand, natural pesticides are the right choice because they are environmentally friendly and have multiple effects, namely, eradicating pests, killing invasive species (Mrozik et al., 2019), and serving as natural fertilizers after a certain period of time (Ahmad, Sheikh Abdullah, Hasan, Othman, & Ismail, 2021). Several local plant species have the potential to control pests in cultivation activities and can be used as pesticides on a large scale because they are biodegradable, food safe, and environment friendly (Ferdous et al., 2018).

One material that has active compounds that can be used as natural pesticides is the fruit of fish poison tree (*Barringtonia asiatica*), which can be locally obtained in Papua and has not been used widely. Utilization of this fruit is an appropriate and innovative measure to reduce the use of synthetic pesticides that harm environmental ecosystems. Fish poison tree fruit contains saponins (Montes De Oca et al., 2017; Umaru, et al., 2018; Natawigena et al., 2018), terpenes, alkaloids, triterpenoids, phenolics, and tannins (Umaru et al., 2019). *Barringtonia asiatica* has several benefits, among others, to treat stomach pain and rheumatism. Its fruit and seeds are used as fish poison. The juice of the fruit is used to treat scabies, while the seeds can also be used as an anthelmintic and the bark is a cure for tuberculosis (Ragasa et al., 2014).

Barringtonia asiatica is a tree that is commonly found along the coast. The fruit of the fish poison tree (B. asiatica) is dispersed by floating at the sea during the rainy season. This plant then thrives and is widely dispersed widely on islands and beaches (Quigley, Gainey, & Dinsdale, 2014). Fish poison tree

(*B. asiatica*) is a tropical tree in mangrove forests whose characteristics are that it can grow on sandy beaches, corals and sand plains or river banks, mangrove swamps, and limestone hillsides and is resistant to salt water. *B. asiatica* is widely distributed from East Africa, Pemba Island, Comoro Islands, Madagascar, Seychelles, Mauritius, Chagos Archipelago, India, Sri Lanka, Andaman Islands, Thailand, Cambodia, to Vietnam (Sourav, 2019) and Indonesia (Rumampuk et al., 2003).

A previous study reported that aqueous crude extract from the seeds of fish poison tree (*B. asiatica*) fruit showed high toxicity and adversely affected the hatchability and lethality of brine shrimp in a test (Mojica & Micor, 2007). In a study, it is also known that methanol extracts and n-hexane, dichloromethane, ethyl acetate, and butanol fractions of the leaves, fruits, seeds, stems, and root bark of *B. asiatica* have antimicrobial activity (Umaru et al., 2019: Umaru et al., 2020). Furthermore, the results of another study on *B. asiatica* revealed that *B. asiatica* contains saponins which are suspected to function as antifeedant against *Epilachna sp.* larvae (Herlt et al., 2002). In addition, Burton et al. (2003) discovered ranuncoside, which is oleanane glycoside that has piscicidal activity, in fish poison tree (Rumampuk et al., 2003).

Triterpenes that were obtained from the extracts of bark, roots, and flowers of fish poison tree (*B. asiatica*) displayed antimicrobial activity. The extracts obtained by conventional cold-water immersion method using nonpolar, semipolar, and polar solvents (hexane, dichloromethane, chloroform, ethyl acetate, and methanol) exhibited antimicrobial activity against fungal pathogenic strains. A testing on fish poison tree has shown that the plant has antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. The result of the minimum inhibitory concentration testing on hexane extract showed a significant inhibition against *Aspergillus niger* at a dose of 500 g/mL with an inhibition zone of 14.77±0.05 mm. In addition, the dichloromethane crude leaf extract showed higher inhibition against the growth of *Fusarium oxysporium* with an inhibition zone of 15.93 ± 0.05 mm (Umaru et al., 2019).

Based on the description of several facts from previous research, it can be said that tropical plant such as fish poison tree has the potential to be used widely as a natural pesticide because it contains saponin compounds. In addition, fish poison tree (*B. asiatica*) exhibits antibacterial, antifungal, and antimicrobial activities (Umaru et al., 2020) because it contains secondary metabolites, including saponins and terpenoids (Montes De Oca et al., 2017; Umaru et al., 2018; Natawigena et al., 2018), terpenes, alkaloids, triterpenoids, phenolics, and tannins (Umaru et al., 2019). Saponins are triterpene and sterol glycosides, having the ability to induce hemolysis in fish and cause poisoning in livestock (Harborne, 1987). The purpose of this study was to determine the most effective dose of saponins that could be used to control pests in ponds based on the results of phytochemical screening test on seeds and peels of mature and raw fruits of fish poison tree (*B. asiatica*) and based on the result of assessment of saponins as potential biopesticide for aquatic pests.

METHOD

The materials used were seeds and peels of raw fruit and mature fruit of fish poison tree (*B. asiatica*), which were obtained from the beach around the campus of the Sorong Marine and Fisheries Polytechnic. Furthermore, this study used tilapias with size ranging from 7 cm to 8 cm that were obtained from the Freshwater Aquaculture Research and Development Installation (IP2BAT) as experimental animals. Before tilapias were used as experimental animals, they were first acclimatized in a controlled vessel for 14 days. Saponins from tea seeds used as control were obtained from commercialized factory processed products.

Phytochemical screening test

Initial screening test is needed to determine the presence of bioactive content in fish poison tree. According to Suhermanto et al. (2013) and Harborne (1987), samples need to be extracted using sterile distilled water as a polar solvent for approximately 24 hours. The filtrate obtained was taken as much as 2 mL for the saponin test.

Test for saponin was done by adding alcohol to the sample. A positive test result is indicated by the presence of permanent foam on the surface. Ninety grams of seed powder, raw fruit peel powder, and mature fruit peel powder were taken each and macerated for 24 hours in 500 mL alcohol. The filtrate was then filtered to obtain an alcoholic extract solution. The solution obtained was then evaporated using a rotary evaporator at a temperature of 40ºC until all the solvent evaporated and a concentrated extract was obtained (Harborne, 1987).

Effectiveness test on the administration of *B. Asiatica* extract

The research design used in this study was completely randomized design (CRD). The treatments in this study were different doses with different ranges and control, all of which were repeated 3 times, as follows:

1. Treatment A (*B. asiatica* seed) : administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm;
2. Treatment B (*B. asiatica* raw fruit peel) : administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm;
3. Treatment C (*B. asiatica* mature fruit peel) : administration of crude extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm;
4. Control (D) : Saponins from tea seeds at a dose of 20 ppm (Shariff *et al.* 2000).

Tilapias were kept in an aquarium with a density of 20 tilapias/20 L. The research activity was conducted during the day to optimize the performance of the crude extracts of *B. asiatica*. Supporting measures that were conducted were physiological observation of external changes in tilapia and measurements of water qualities, including temperature, pH, and dissolved oxygen (DO), after the treatments were given.

Data analysis

The data obtained from the results of the study were statistically analyzed with one-way analysis of variance (ANOVA) with the help of Minitab Statistical Software version 16 to determine the differences between treatments. Data analysis was then continued with Fisher's extract test. The advantages of *B. asiatica* were described descriptively. The economic value was known by calculating the cost of using saponins in controlling pests in one cycle of whiteleg shrimp cultivation.

Results and Discussion

Fish poison tree (*B. asiatica*) is a plant known with various names, for example, *boton* in the Philippines, *butun*, *putat laut*, *bitung*, and *keben* in Indonesia, and box fruit in India. Fish poison tree is a species of mangrove plant whose habitat is spread on tropical beaches and islands in the Pacific Ocean, India, Zanzibar, Taiwan, Philippines, Fiji, New Caledonia, Solomon Islands, Cook Islands, Wallis and Futuna Islands, and French Polynesia (Quigley et al., 2014). This plant likes a wet and humid tropical climate. Its stem growth is episodic and it shows segmentation between the axes with prominent leaf scars. Leaf size varies from small to medium lanceolate leaves 5–20 cm wide in leptocaul species to large ovate-lanceolate leaves 20–40 cm wide (Figure 1).

Figure 1. Leaves, fruit, and seeds of (*B. asiatica)*

Preliminary Test

The formation of long-lasting foams when a plant is extracted or when a plant extract is concentrated is evidence of the presence of saponins (Harborne, 1987). Saponins can be detected in crude extracts based on their ability to induce hemolyis. The results of the preliminary screening of the saponin content in *B. asiatica* can be seen in Table 1.

Table 1. Results of preliminary screening of fish poison tree (*B. asiatica*)

Sample	Solvent	Test result	Description*
Seed raw fruit peel mature fruit peel	HCl	permanent foam	saponin compounds (++) saponin compounds (+) saponin compounds (+)
Seed raw fruit peel mature fruit peel	Alcohol	permanent foam	saponin compounds (++) saponin compounds (+) saponin compounds (+)

*2 mL sample, 0.1 mL solvent

The filtrate that was reacted with concentrated HCl and alcohol gave positive results, indicated by the formation of permanent and dominant foam. Suhermanto et al., (2013) state that if the addition of 3 drops of concentrated HCl into the extract causes the formation of permanent foam, it indicates the content of saponin compounds.

Phytochemical screening test results

The results of phytochemical screening test on fish poison tree indicated the presence of phytochemicals. This can be seen in Table 2.

Table 2. The results of phytochemical screening test on fish poison tree

Extract	Test	Result	Description
Seed	Alkaloid	++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
	Flavonoid	-	No color change
	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change
Raw fruit peel	Alkaloid	++++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
	Flavonoid	++++	Reddish sample solution
	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change
Mature fruit peel	Alkaloid	++++	There is a precipitate during the test with Dragendroff's reagent and the sample solution changes color to milky white
	Flavonoid	++++	Reddish sample solution
	Saponin	++++	There is a lot of foam and lasts for 10 minutes
	Tanin	++++	The sample solution changes color to black
	Steroid/Triterpen	-	No color change

The results of the phytochemical screening test showed that all components of fish poison tree contained alkaloids, flavonoids, saponins, and tannins. The yield of fish poison tree seeds of 2.488% contained saponins of 1 g, the peel of raw fruit of fish poison tree with a yield of 0.455% had a saponin content of 0.06 g, and the peel of mature fruit of fish poison tree with a yield of 2.122% had a saponin content of 0.07 g.

Test on the effect of saponins on Tilapia

This test was conducted to determine the effect and effectiveness of saponins from crude extracts of seeds and peels of raw fruit and mature fruit of fish poison tree in controlling pests using tilapia as test animal. The results of the saponin test on tilapia can be seen in Table 3.

Table 3. The results of test on the effect and effectiveness of saponins on tilapia

Sample	Dosage (ppm)				Description
	10	20	30	40	
Seed	+	+	+	+	Tilapia is dead
raw fruit peel	-	-	-	-	Live tilapia
mature fruit peel	-	-	-	-	Live tilapia

Positive values were shown by the seed extract with doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm, where a dose of 40 ppm was lethal to tilapia. The effect of the administration of saponins derived from fish poison tree seeds on the time of death of tilapia can be seen in Table 4.

Table 4. The effect of the administration of saponin compounds on the time of death of tilapia

Dosage	Replication			Average (minute)	Avarage±Stdev
	1	2	3		
10	118.8	108.8	108.4	112.00	112±5.89
20	92.9	92.9	91.5	92.43	92.43±0.80
30	51.5	48.6	47.0	49.03	49.03±2.28
40	36.5	42.2	39.2	39.30	39.30±2.85

The results of data analysis using one-way analysis of variance (ANOVA) discovered that the administration of saponins at doses of 10 ppm, 20 ppm, 30 ppm, and 40 ppm was significantly different between treatments at the 5% level. Saponins displayed other toxic activities, namely, interfering with the regulation of ions and osmotic pressure. This compound can bind oxygen so that oxygen levels in water decrease (Das et al., 2017). This decrease in oxygen levels has a negative impact on fish.

Saponin acts as hemolytic agent. It breaks the bonds of hemoglobin molecules and is responsible for the rupture of the erythrocyte bio-membrane. Phyto-saponins from the seeds of tamarind (*Tamarindus indica*), husks, and sugarcane jaggery are hemotoxic to fish (Das et al., 2017). Phyto-saponins are widely used in traditional aquaculture in Asia and Africa for pest control because of their low toxicity to mammals (Clearwater et al., 2008).

Saponins are by-product of the manufacturing process of tea seed oil (from *Camellia sinensis*) and are commonly used as organic pesticides in ponds. Tea seeds contain 10-15% saponins, which are effective in controlling pests, but are not lethal to shrimp. Saponins are used as initial and additional pesticides in monoculture and polyculture systems. The dose of saponins commonly used in ponds ranges from 15 to 25 ppm. The higher the salinity is, the lower the dose of saponins applied becomes. Saponins also play a role in stimulating molting in cultured shrimp (Ng'ambi et al., 2017), and are used as organic fertilizers that can stimulate algae growth in ponds (Castillo-Ruiz et al., 2018).

Saponins contained in organisms generally act as toxic agents to protect the organisms from predators. Invasion of foreign material that is not detected by antigen results in damage to hematopoietic organs so that hemoglobin (Hb) decreases (Suhermanto et al., 2011). Changes in hemoglobin content are positively correlated with a decrease in the number of erythrocytes, which is caused by hypochromic microcytic anemia due to infection by saponins. The decrease in hemoglobin concentration is also caused by leukocytosis, which in turn has an impact on the occurrence of erythroblastosis (Harikrishnan et al., 2003)

Low hemoglobin causes the metabolic rate of fish to decrease so that the energy produced by fish is low. This condition will have a negative impact on fish. Fish will float on the surface of the water and eventually die (Suhermanto et al., 2011). Damage to erythrocytes will also cause disruption of oxygen transport to fish body tissues, thereby inhibiting metabolic processes (Suhermanto et al., 2013).

Saponins are harmful to fish, but at certain doses, they have a positive impact on biota. (George Francis, Makkar, & Becker, 2002) revealed that saponins from quillaja that were mixed in feed at a dose of 150 mg per one kilogram of feed could increase the growth of common carp (*C. carpio*) by 372%, while the control increased the growth of common carp by 327%, and treatment of 300 mg per one kilogram of feed increased the growth of common carp only by 325%. (George Francis, Makkar, & Becker, 2005) states that saponins derived from quillaja that are then mixed in feed can increase the growth, metabolism, and reproduction of tilapia. Quillaja mixed in feed does not affect the egg production of tilapia, but instead it can increase its metabolism and growth was 245% over the initial body mass, higher than that of the control group 188% (George Francis et al., 2001).

Research conducted on the peel of raw fruit and mature fruit of fish poison tree indicated that saponins did not have a lethal effect on fish. Administering saponins extracted from fish poison tree to tilapia is suspected to be able to improve the immune response in tilapia. Administration of low and high doses of saponins will be tolerogenic, while the optimum dose can increase the immune response. Doses that are too high can interfere with fish's immune system and will cause damage to organs and cells with the mechanism being unable to tolerate conditions beyond its capabilities.

Foreign substance that enters the body of a biota will be identified in such a way that it is recognized, then it will be processed and sent to the cell-mediated antibody-forming system. Lymphocytes are cells that specifically recognize and respond to external antigens and then act as mediators between cell-mediated immunity and humoral immunity. B lymphocytes are cells with the ability to produce antibodies, recognize extracellular antigens, and differentiate between antibodies in plasma cells. T cells are intermediate immune cells that recognize intracellular antigens and function to destroy microbes or infected cells (Suhermanto et al., 2011)

Water quality

The results of measurements of water temperature in the aquariums showed a temperature range between 29ºC–31ºC. Temperature greatly affects the rate of reaction of saponins. The higher the water temperature, the faster the saponins will kill pests. The measurements of the pH of the water during the study obtained a pH value between 7.3 - 7.5, while the measurement of dissolved oxygen in the aquariums showed the results ranging from 6.9 mg/L to 7.3 mg/L. The parameters above are still within the optimal range for tilapia maintenance based on SNI 7550:2009.

The advantages of using fish poison tree in aquaculture

Saponins as natural compounds are obtained from tropical plants (Quigley et al., 2014) and are used in shrimp culture at the land preparation stage to eradicate pests such as wild fish that become predators, competitors, and nuisances in ponds. Commercial saponin products that are widely used are usually made from the seeds of tea (*Camellia Oleifeira*). However, tea seeds still have to be imported and are expensive in Papua. Fish poison tree as an alternative source of saponins has proven to be effective and can be used to support environmentally friendly and sustainable aquaculture, as can be seen in Table 5.

Saponins from the seeds of fish poison tree (*B. asiatica*), referring to the results of the study presented above, have the potential to function as invasive fish control. However, saponins in general are also useful as antimicrobial agent, immunostimulant, and surface tension reducer (Francis et al., 2005)

The economic value of the use of saponins derived from fish poison trees for whiteleg shrimp farmers in Sorong, West Papua, can be identified by comparing the use of saponins in the cultivation of whiteleg shrimp. The saponins needed in pond preparation for aquaculture are around 50 kilograms per production cycle, with a price of IDR 35,000 per one kilogram of saponins. Thus, it can at least save production costs of around IDR 1,750,000 for each plot with an area of about 1,700 m^2 and a pond depth of 1-1.5 m. Another advantage of the use of saponins derived from fish poison tree as biopesticides is that these saponins are local raw materials so they are easy to obtain and low-cost, thus reducing operational costs, and it supports organic farming practices (Ahmed et al., 2020) that are safe for cultivated biota (shrimp). In addition, saponins do not pollute waters and biota that are produced from aquaculture that uses saponins as pest control is safe for consumption and good for consumer health.

Table 5. Comparison of saponin applications in land preparation

Ingredients	Dosage (ppm)	Price/kg (IDR)	Description
Seed of tea (*C. Oleifeira*)	15-20	35,000	Impor
Seed of Keben (*B. asiatica*)	10-15	0	Local raw materials

CONCLUSION

The results of the phytochemical screening test revealed the presence of secondary metabolites in fish poison tree (*B. asiatica*), including saponins, alkaloids, flavonoids, and tannins, with the highest concentration of bioactive saponins found in seeds. Administration of crude extract of fish poison tree (*B. asiatica*) seeds to test animals showed a positive impact at doses starting at 10 ppm. The higher the dose of saponin, the faster the saponin will eradicate pests. Saponins from fish poison tree seeds have potential benefits as invasive fish repellents. The use of saponins obtained from the seeds of fish poison tree (*B. asiatica*) has economic value because it is from local materials, is environmentally friendly, and is safe for human health.

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