Aquacultura Indonesiana 2022 23(2): 118-138
ISSN : 2477-6939

ANTIPARASITIC ACTIVITY OF *Solanum ferox* EXTRACT AND CONCOCTION WITH *Zingiber zerumbet* AND *Boesenbergia pandurata* EXTRACTS TO CONTROL *Argulus* sp. ON GOLDFISH (*Cyprinus carpio*)

Esti Handayani Hardi[1,2*], Rudi Agung Nugroho[2,3], Irawan Wijaya Kusuma[2,4], Gina Saptiani[1,2], Agustina[1], Abinawanto[5], Yushinta Fujaya[6], Bambang murtiyoso[7], Tjahjo Winanto[8], Nurhidayat[9]

[1]*Microbiology Laboratory of Fisheries and Marine Sciences, Department of Aquaculture, Fisheries and Marine Sciences, Mulawarman University, Indonesia.*
[2]*Research Centre of Medicine and Cosmetics in Tropical Rainforest Resources, PUI-PT OKTAL, Mulawarman University, Samarinda, Indonesia*
[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan, Indonesia.*
[4]*Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia.*
[5]*Departement of Biology FMIPA, Universitas Indonesia.*
[6]*Faculty of Marine Science and Fishery, Hasanuddin University, Makassar, Indonesia.*
[7]*Politeknik AUP, Jakarta*
[8]*Faculty of Fisheries and Marine Science Jenderal Soedirman University*
[9]*Research Ornamental Fish Culture, KKP, Indonesia*
*Corresponding authors, e-mail: estie_hardie@fpik.unmul.ac.id

Abstract

Argulus is an ectoparasite that frequently infects goldfish, with severe effects and a high death rate. The use of plant extracts in this research were *Solanum ferox* extracts/SFE (400 ppm), *Boesenbergia pandurata* extract/BPE (900 ppm) and *Zingiber zerumbet* extract/ZZE (200 ppm) have been widely reported to have antiparasitic activities. This research evaluated the antiparasitic activities of compositions A (SFE and ZZE with ration 1:1), B (SFE and BPE with ratio 1:1), and C (single SFE). The research stages comprised 1) isolation and analysis of solasodine content in crude extract of SFE and in three composition A, B, and C, 2) *in vitro* antiparasitic activities on *Argulus* sp. at doses of 50 and 100 mL of each composition in 100 mL water with a testing time of 60–240 minutes, and 3) *in vivo* antiparasitic activities on *Argulus* sp. on goldfish at a dose of 100 mL each composition in 100 mL water and observation for 2–12 hours of fish treatment. The results showed that SFE crude extract had a solasodine concentration of 7.151 mg/L, whereas the solasodine in composition A (656 ppm), B (485 ppm), and C (295 ppm), respectively. The *in vitro* testing demonstrated that composition A was effective for killing approximately 80–100% of *Argulus*, whereas compositions B and C killed 80–90% and 60–70%, respectively. Viewed from the effectiveness for killing parasites, the result was excellent (above 50%). The *in vivo* treatment test was continued using three extract compositions at a dose of 100 ppm. Compositions A, B, and C were found to be capable of releasing 81.33, 75.67, and 71.00 arguli, respectively, per fish. We concluded that the single SFE extract and a concoction with BPE and ZZE had reasonable antiparasitic activity, whereas the concoction of SFE and ZZE killed more *Argulus* parasites at a higher rate.

Keywords: antiparasitic, *Argulus* sp, fish diseases, natural product, *Solanum ferox*, Solasodine

Introduction

Argulus japonicus was found first in Asia to infect goldfish (*Carassius auratus*) and koi fish (*Cyprinus carpio*). It has also been reported in the United States of America, particularly in Florida, Georgia, Louisiana, California, Hawaii, Illinois, Maryland, Washington, and Wisconsin (Kipp *et al*. 2014). The expanding infection has been caused by the transportation of koi fish, as the carrier of *Argulus* sp., although this fish might be infected by other pathogens, namely bacteria, fungi, and parasites (Al Darwesh *et al*. 2014).

According to Steckler and Yanong (2012), *Argulus* sp. belongs to the Branchiura parasite and has an oval shape, covered by a wide carapace, sunken on the adhesive side, and convex on the other. *Argulus* sp. has two compound eyes, and its body has a suction disc that makes it is difficult to clean with water, although it can be removed with abrasion. Forcibly removing the parasite results in a wound on the fish skin because *Argulus* has a venom gland on its stylet that is used for penetrating the skin, transferring venom into the blood and body liquids. It causes skin irritation and visible inflammation, namely redness and wound on the infected skin (Noga 2000; Kabata 1985; Alom *et al.* 2019). The *Argulus* sp. parasite is frequently found in freshwater fish, sea fish, crab, lobster, and shrimp. There are almost 100 *Argulus* species worldwide, infecting several hosts (Steckler & Yanong 2012).

The *A. japonicus* parasite, infecting the caudal and anal fins, is found on the body surface, whereas operculum is also found in the gills of goldfish (*Carassius auratus*). *Argulus* in fish can cause bleeding, wounds, and ulcers, and the formed wound can be a location for pathogens (Aeromoniasis, Pseudomoniasis) to penetrate (Noaman *et al*. 2010). Furthermore, according to Lamarre and Cochran (Lamarre & Cochran 1992), fish infected with *Argulus* show abnormalities in behavior, including irritation, color change, listlessness, anorexia, and anemia because the pre-oral stylet that stabs the fish skin has a cytolytic toxin that causes damage to blood cells, mucus, and epithelial cells, softening the fish and allowing *Argulus* to more easily consume it.

The death rate in infected fish is relatively high. Therefore, this organism causes considerable loss in both small and large fish. To solve the issue of *Argulus* sp., many farmers use chemicals, such as sodium chloride[9] and trichlorfon at 0.25 ppm (Noaman *et al*. 2010). However, antibiotic or chemical use can cause irritation on fish skin, and side effects associated with the use of chemicals on the environment are frequently reported (Pandey *et al.* 2011). Although the utilization of chemicals on decorative fish is allowed, their continuous use negatively affects fish health.

The use of natural materials for hampering and curing infected fish has been widely reported

Tona *et al.* (1998), Willcox and Bodeker (2000), Asres *et al.* (2001), Satrija *et al.* (2001), Ijah and Oyebanji (2003). Extract of garlic (*Allium sativum*) is capable of killing trichodinids (Madsen *et al.* 2003), theronts, and tomonts from the *Ichthyophthirius multifiliis* parasite (Buchmann *et al.* 2003). Ekanem *et al.* (2004) used extracts from *Mucuna pruriens* and *Carica papaya* to kill the free-swimming parasite, *I. multifiliis*. One of the potential antiparasitic plants is sour eggplant, which contains solasodine, a steroidal glycoalkaloid compound. Some studies have shown that solasodine has antifungal (Ekanem *et al.* 2004; Wang *et al.* 2000), antibacterial (Mandalakis *et al.* 2021; Kumar *et al.* 2009; Bibon 2021; Jubair *et al.* 2021; Hardi *et al.* 2021; Hardi *et al.* 2022), anticancer (Shen *et al.* 2017; Xu *et al.* 2017; Khandani *et al.* 2019; Singh *et al.* 2019), and antiparasitic potencies as well as several other important activities (Krieg *et al.* 2016).

This article discusses the detection of solasodine compounds in the extracts of sour eggplant and the use of single *Solanum ferox* (SFE) extract and a concoction with *Boesenbergia pandurata* (BPE) and *Zingiber zerumbet* (ZZE) extracts to kill and release *Argulus* sp. that infects goldfish (*C. carpio*). These three extracts have been widely found to have antibacterial effects against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Vibrio harveyi*, and *V. haemolytic* (Hardi *et al.* 2016a; Hardi *et al.* 2016b; Hardi *et al.* 2018a; Hardi *et al.* 2018b; Hardi *et al.* 2018c; Hardi *et al.* 2021; Hardi *et al.* 2017; Hardi *et al.* 2019; Hardi *et al.* 2019; Hardi *et al.* 2021; Hardi *et al.* 2022). Various contents (e.g., alkaloids, flavonoids, solasodine, zerumbet, and panduratin) are secondary metabolic materials in these three extracts capable of negatively affecting pathogens (e.g., bacteria, fungi, protozoa, crustacea, nematodes).

The first case of the *Argulus* parasite was reported in the Mahkota fish pond, Sungai Kapih Street, Sambutan District, Samarinda City, East Kalimantan on the 20th of February 2021. The owner of this fish pond contacted the Laboratory of Aquaculture Microbiology and Biotechnology, Faculty of Fishery and Marine Sciences on the 24th of February 2021. Observations regarding fish death since the 20th of February 2021 showed that 20 of 130 goldfish in the pond died; it was reported later that 102 fish died. After the reporting, the medication process was initiated using SFE, BPE, and ZZE in single and mixed extracts with the immersion method. The medication process is further described in the next chapter.

The purpose of this research is to evaluate antiparasitic activity of *Solanum ferox* (SFE), *Zingiber zerumbet* (ZZE), and *Bosenbergia pandurate* (BPE) extracts for prevention and treatment of *Argulus* sp. in goldfish (*Cyprinus carpio*).

Materials And Methods

1. Experimental Fish

Goldfish (*Cyprinus carpio*) with a weight of 4–5 kg (individual weight) were obtained from the Mahkota fish pond, Sungai Kapih Street, Sambutan District, Samarinda City, East Kalimantan. Meanwhile, 36 fish severely infected by *Argulus* were used and were marked with redness and *Argulus* found throughout the entire fish body, including the caudal, pectoral, and abdominal fins, at a high percentage. An experiment was conducted on the 24th of February 2021 during one death case caused by the attack of *Argulus*.

2. Plant extracts and Extraction process

The third extraction process of material was conducted in stages that were almost the same as the method of Hardi *et al*. (2016a) and Hardi *et al.* (2016b), in which BPE, ZZE, and SFE were washed until clean with flowing water, cut into small pieces with a thickness of 0.5 cm using a cutting machine, and dried with an oven (40–45 °C) for 24 hours for temu kunci and lempuyang and 48 hours for sour eggplant. After that, the dried ingredients were mashed with a blender, and the simplicia slices were soaked in 96% ethanol liquid, filtered with filter paper, and evaporated using an evaporator with scale-up. The extracts were transferred into a sterile Becker glass, stored in an oven for 24 hours to obtain an extract viscosity of 10–11, and then maintained at 4 °C before use. The extracts used in this research had two compositions, using the best concentration of three extracts from previous research (Hardi *et al.* 2016a; Hardi *et al.* 2016b; Hardi *et al.* 2018a; Hardi *et al.* 2018b; Hardi *et al.* 2018c; Hardi *et al.* 2021; Hardi *et al.* 2017; Hardi *et al.* 2019; Hardi *et al.* 2019).

The experiment medication of *Argulus* sp. in goldfish were used three different extract compositions, namely:

Composition A : SFE 400 ppm and ZZE 200ppm extracts with ratio 1:1.

Composition B : SFE 400 ppm and BPE 900 ppm extracts with ratio 1:1.

Composition C : A single of 400 ppm SFE extract.

3. Solasodine content analysis

HPLC was used for determining the solasodine content in the sour eggplant extract and its derivative product. The condition/system of analysis for extract sample, extracts composition A, B, C using HPLC was as follows:

Motion Phase	:Acetonitrile
(30:70) 0.5% phosphoric acid	
Injection Volume	: 10 µL
Column	:ShimPack
GIST C18	
Column Oven Temperature	: 25 ^{o}C
Solvent Flow Rate	: 1 mL/minute
Detector	: PDA
Analysis Time	: 10 minutes

Solasodine contents were determined at the Advanced Characterization Laboratory of Cibinong – Integrated Bioproduct Laboratory,

National Institute of Science. Examinations of solasodine bioactive compounds were performed in *S. Ferox* extract and Compositions A (SFE and ZZE), B (SFE and BPE), and C (SFE) from the extract used in this research. Solasodine can be used as one of the identifiers for determining plant identity in *Solanaceae* and its extract. Content in plant extract from the *Solanaceae* family on its derivative products can be approached with the solasodine tracking method.

4. I*n vitro* testing of parasitic activities from plant extracts

In vitro analyses were conducted following the procedure of Ekanem *et al.* (2004). *Argulus* sp. were removed from the infected fish carefully with plastic forceps and placed into a petri dish, and moving active parasites were chosen with an ose needle. Ten living, active parasites were moved manually into a petri dish containing 20 ml of liquid extract compositions A, B, and C at different concentrations. Every 60 minutes, the number of dead parasites was calculated until 240 minutes. Parasite death was determined when *Argulus* sp. did not show any motion after being touched with the ose needle.

This *in vitro* research used seven treatments, and every treatment was repeated three times as follows:

For the control, *Argulus* was soaked with freshwater without adding extract/0 ppm.

A1, *Argulus* was soaked with composition A at 50 mL in 100 mL water.

A2, *Argulus* was soaked with composition A at 100 mL in 100 mL water.

B1, *Argulus* was soaked with composition B at 50 mL in 100 mL water.

B2, *Argulus* was soaked with composition B at 100 mL in 100 mL water.

C1, *Argulus* was soaked with composition C at 50 mL in 100 mL water.

C2, *Argulus* was soaked with composition C at 100 mL in 100 mL water.

The antiparasitic efficacy of each group was calculated using the equation of Wang *et al*. (2000):

AE = (B -T)/B X (100%)

in which AE=antiparasitic efficacy, B is the mean number of surviving *Argulus* in the control, and T is the mean number of surviving *Argulus* in the treatment.

5. *In vivo* testing of parasitic activities from plant extracts on goldfish

This stage involved medication using four treatments, and every treatment was repeated three times. The *In vitro* testing showed that the single extract and concoction with three extracts had antiparasitic capabilities against *Argulus*, but 100 ml had better antiparasitic activities, compared with 50 ml; hence, the *in vivo* tests used four treatments and three repetitions, as follows:

K treatment, fish soaked with freshwater without adding extract/0 ppm.

A2, *Argulus* was soaked with composition A at 100 mL in 100 mL water.

B2, *Argulus* was soaked with composition B at 100 mL in 100 mL water.

C2, *Argulus* was soaked with composition C at 100 mL in 100 mL water.

Three goldfish (for every repetition) weighing 4–5 kg, infected by *Argulus*, were soaked in extract liquids of different compositions for 12 hours, and the number of *Argulus* released from goldfish was calculated every 2 hours (2, 4, 6, 8, 10, and 12 hours). After soaked with extracts, the fish were reared in static water in ponds measuring 3 × 2 m^2.

After medication for 12 hours, fish were transferred back into the container to evaluate the survival of goldfish for seven days (from the 24th of February 2021 to the 2nd of March 2021). Data regarding fish survival after medication were obtained every day.

6. Statistical analysis

The data were tabulated using Windows Microsoft Excel and were analyzed using Analysis of Variance (ANOVA). To identify the difference between treatments, the data were analyzed using Duncan's new multiple range test. The collected data were analyzed using the statistical package SPSS version 16.

Results And Discussion

1. Analysis of solasodine content in *solanum ferox* extracts and its extracts composition.

A. Solasodine Calibration Curve

A solasodine calibration curve was made as a reference for determining the solasodine level in SFE and the composition of the produced extract. The calibration curve was constructed using a high-performance liquid chromatography (HPLC) instrument with a standard compound concentration of solasodine at 120–600 ppm, resulting in a curve with equation y = 0.0004 + 0.2702 (Figure 1) and correlation coefficient 0.9724. HPLC analysis was showed that the peak for solasodine compound at retention time was 2,379 minutes.

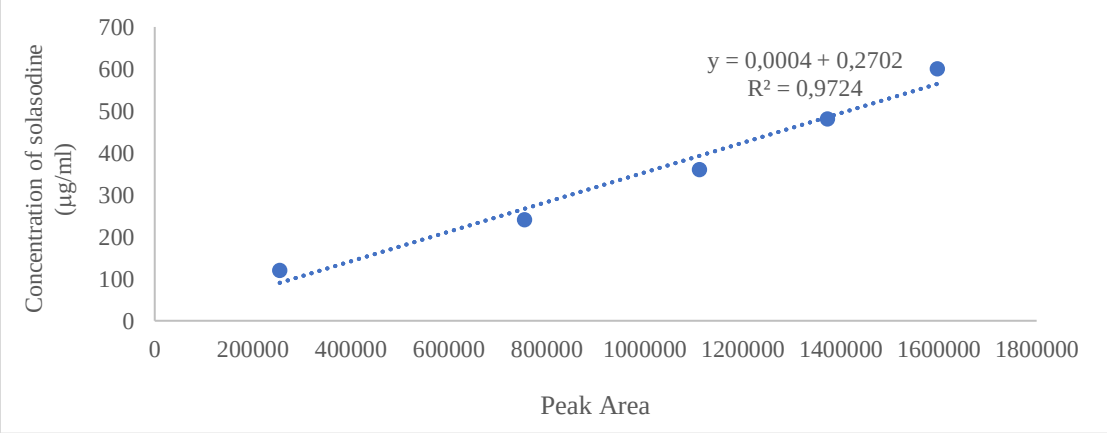


Figure 1. standard calibration curve of Solasodine compound

B. Analysis of solasodine content in *Solanum ferox* crude extracts

Analysis of solasodine content in *S. ferox* fruit extract using High-Performance Liquid Chromatography (HPLC) were discussed in this section. The presence of solasodine were analyzed based on the extracts obtained from *S. ferox* fruit collected from two extraction period (coded with SFE 1 and SFE 2).

HPLC analysis on SFE 1 and SFE 2 were presented in Fig 2. HPLC chromatogram showed at least 12 peaks at retention time ranged from 2.212 to 9.650 minutes. Peak at 2.212 min was identified as solasodine referred to the HPLC analysis of the standard solasodine.

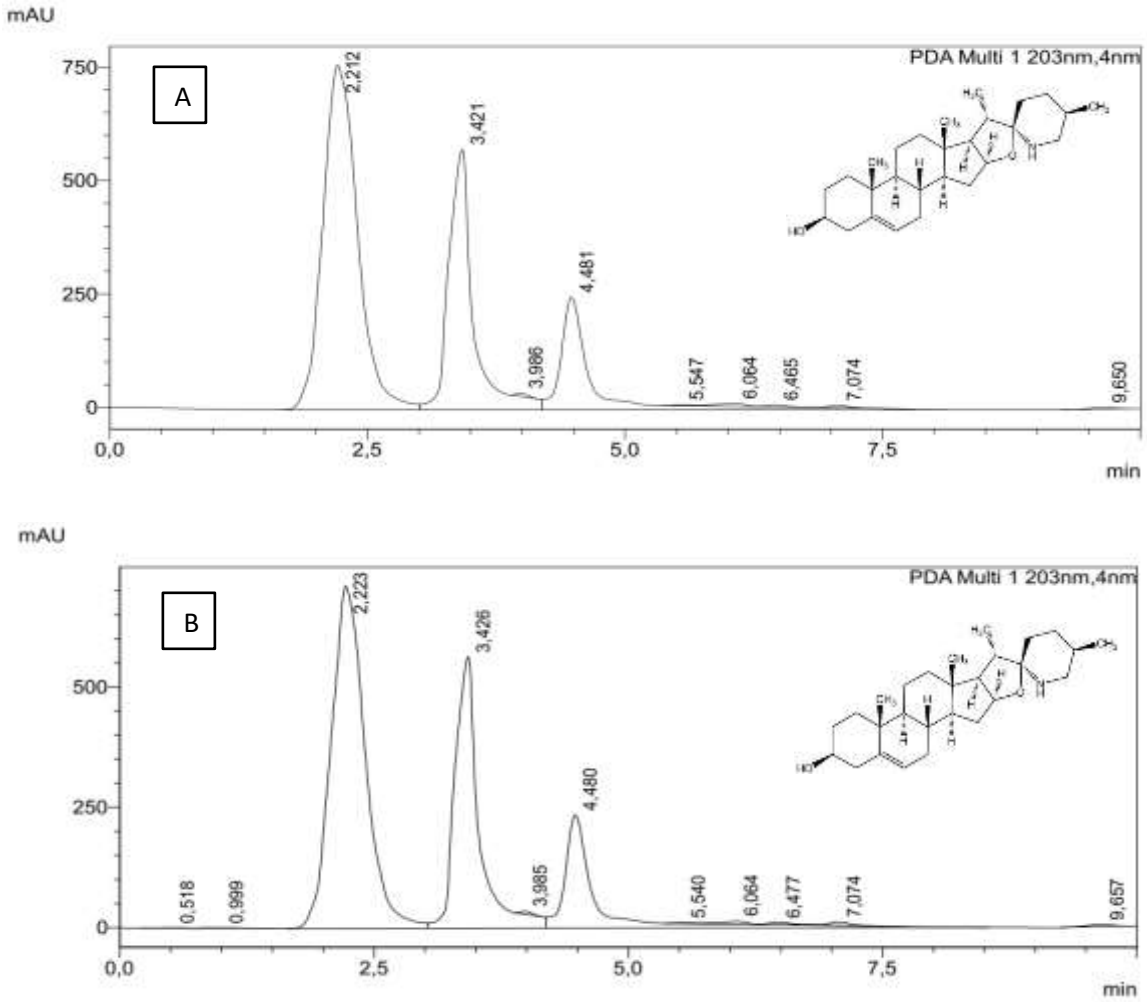


Figure 2. HPLC chromatogram of SFE 1 (A, tR 2.212 min) and SFE 2 (B, tR 2.223 min) showing the occurrence of solasodine in sour eggplant extract

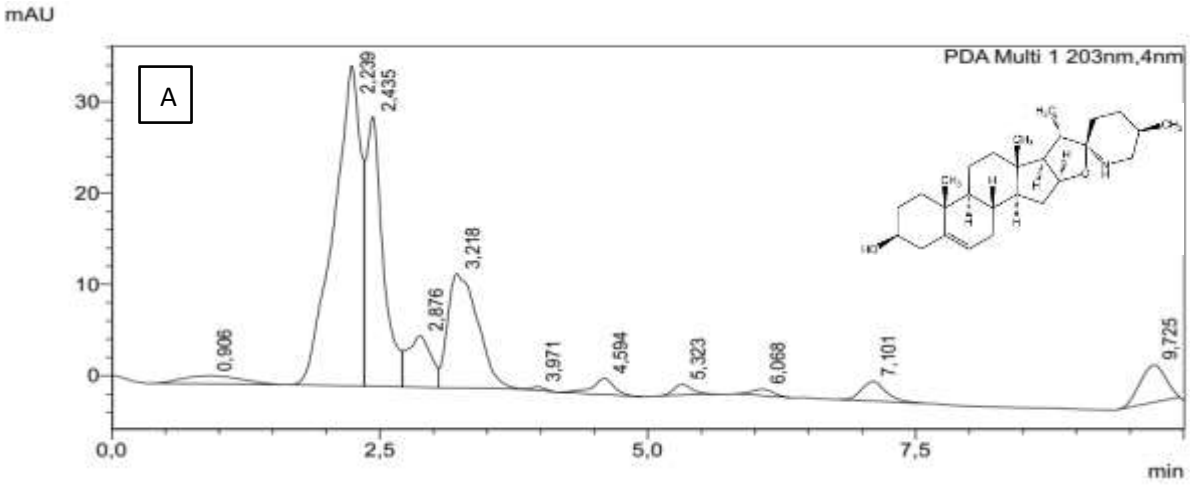
The HPLC chromatogram showed the width of area for the peak at 2.212 minutes (the peak for solasodine) was 17,877,468.

Based on the standard calibration curve of solasodine, solasodine content in SFE 1 was determined 7,151 μg/ml from the basis of 10,000 μg/ml of injected volume in HPLC analysis.

The HPLC analysis of eggplant extract from the extraction period 2 was presented in Fig 2. The HPLC chromatogram showed at least 11 peaks at retention times 0.518 to 9.657 minutes. The peak at 2.223 minutes was confirmed as solasodine by with reference to the HPLC analysis to that of standard solasodine. The HPLC chromatogram showed the width of area for the solasodine peak identified at 2.223 min was 16,594,359. The standard calibration curve suggested the solasodine content was 6,638 μg/ml based on the 10,000 μg/ml of sample volume used in HPLC analysis.

C. Analysis of solasodine content in Composition A (SFE and ZZE)

The *S. ferox* extracts were used as main ingredients of the products composition coded with products A1 and A2. HPLC analysis of product composition A1 was shown in Fig 3. The HPLC chromatogram showed at least 11 peaks at retention times of 0.906 to 9.725 minutes. Solasodine presence in product composition A1 was indicated by the peak at 2.239 min.



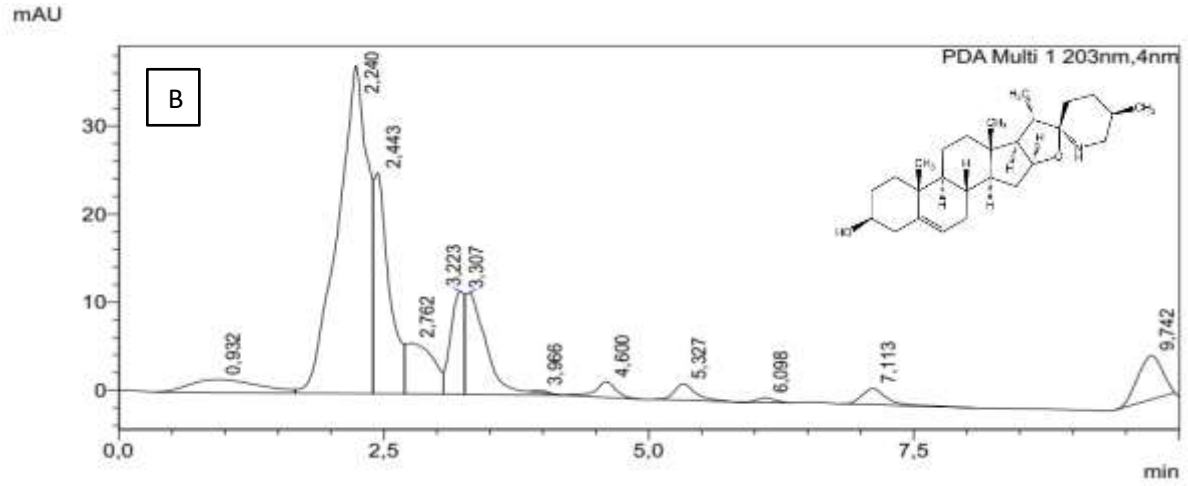


Figure 3. HPLC chromatogram of products A1 (A, tR 2.239 min) and A2 (B, tR 2.240 min) showing the occurrence of solasodine in sour eggplant extract

The HPLC chromatogram showed a peak 2.239 min that is identified as solasodine with the width of area was 622,008. Based on the solasodine calibration curve, the solasodine content in the product A1 was calculated as much as 248 μg/ml.

Furthermore, solasodine content in product composition A2 done by an HPLC analysis was given in Fig 3. HPLC chromatogram provided at least 12 peaks at the retention times 0.932–9.742 min. A peak observed at 2.240 min was determined as solasodine with reference to the standard solasodine analysis. The HPLC chromatogram showed the width of area for the solasodine peak at 2.240 min was 736,862 unit. The solasodine concentration in product A2 based on the equation in the solasodine calibration curve (Figure 1) was calculated to be 295 μg/ml.

D. Analysis of solasodine content in composition B (SFE and BPE)

Other products with the main ingredients of S. ferox extracts were product composition B and coded with product B1 and B2. The HPLC analysis of product B1 was shown in Figure 4. The HPLC chromatogram showed at least 12 peaks from 0.384 to 9.620 min. Solasodine compound in product A was indicated by the occurrence a peak at 2.350 min.

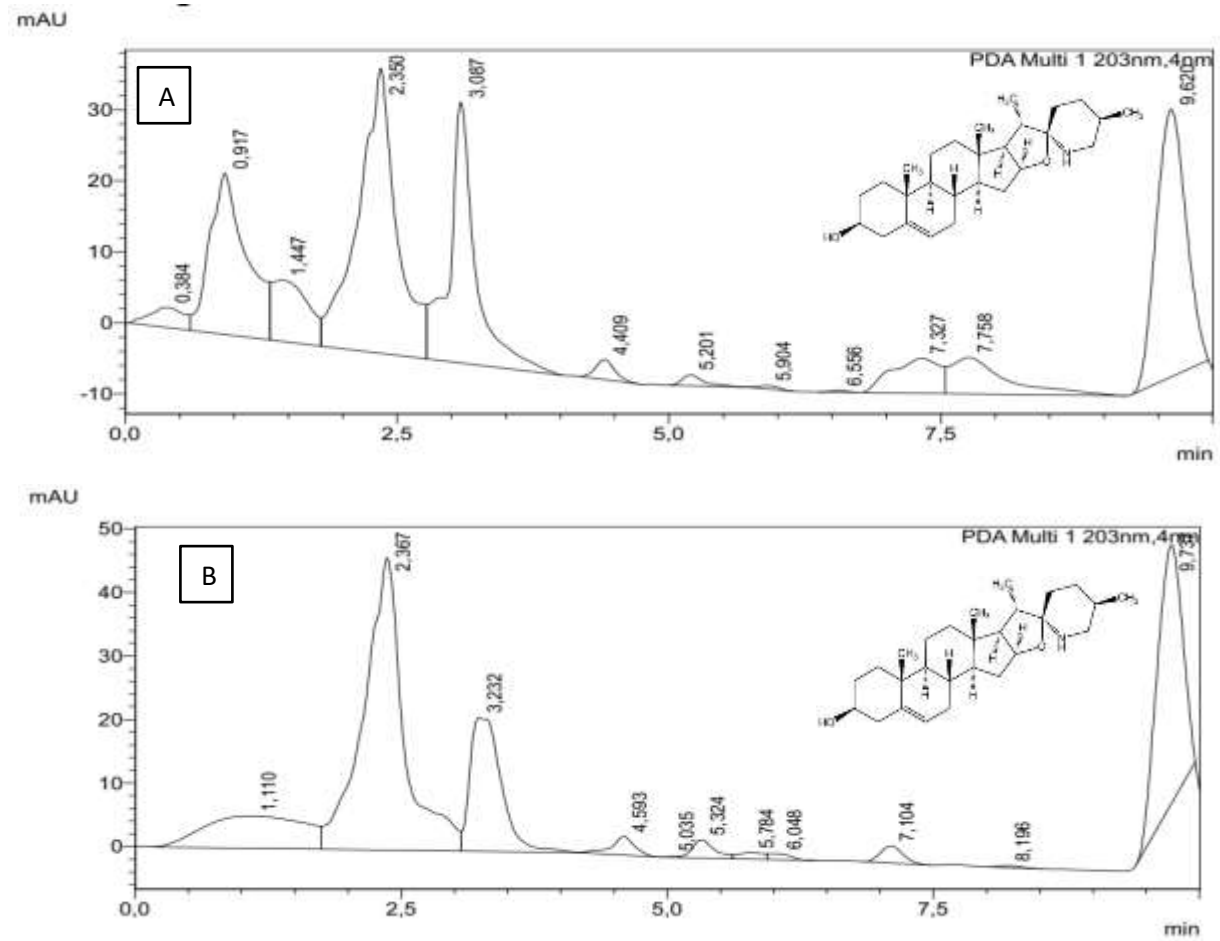


Figure 4. HPLC chromatogram of products B1 (A, tR 2.350 min) and B2 (B, tR 2.367 min) showing the occurrence of solasodine in sour eggplant products.

The HPLC chromatogram showed the width of area for the peak at 2.350 min for solasodine was 981,938). The solasodine content in product B1 was determined as much as 393 μg/ml based on the 10,000 μg/ml of injtected volume of the product.

Solasodine content in product B2 was analyzed by HPLC as shown in Figure 4. Eleven peaks were presenced with the retention times of 1.110 - 9.737 min. Solasodine occurence in product B2 were indicted by the peak appeared at 2.350 min.

A peak at the retention time of 2.367 min having width area of 1,212,706 informed the presence of solasodine. Solasodine content in composition product B 2 was determined as high as 485 ppm with the reference to that of solasodine standard calibration curve.

E. Analysis of solasodine content in composition C (SFE)

Different product composition of S. ferox extracts were prepared and coded as products C1 and C2. The HPLC analysis of product C1 was presened in Figure 5. More than 12 peakswith the retention from 0.975 to 9.740 min were detected at the chromatogram

.

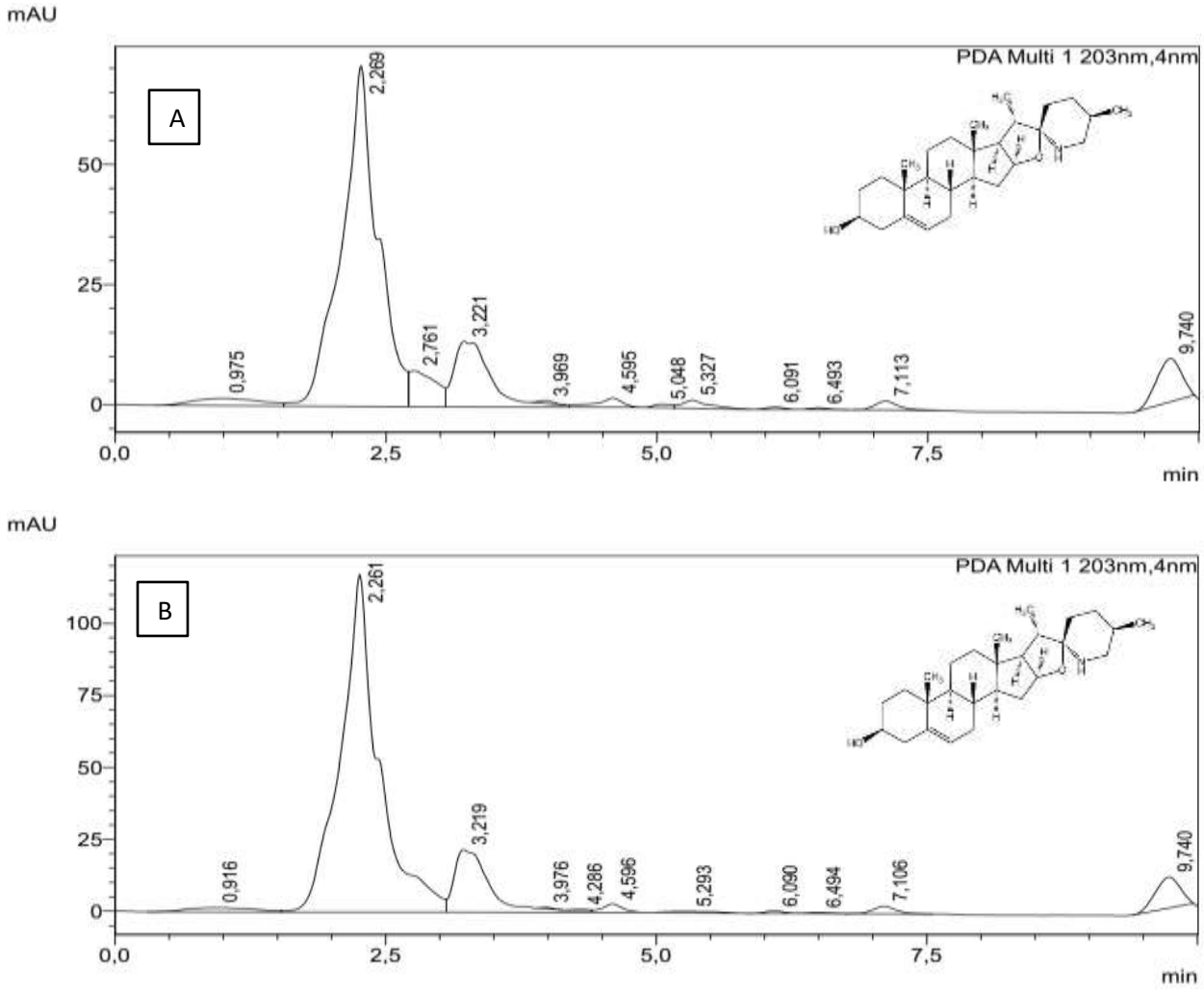


Figure 5. HPLC chromatogram of products C1 (A, tR 2.269 min) and C2 (B, tR 2.261 min) showing the occurrence of solasodine in sour eggplant products

The presence of solasodine in product C1 was indicated by the peak at 2.269 min as presented in Figure 5. min. A peak detected at the retention time of 2.269 min having width area of 1,640,862 was used to calculate the solasodine content. The solasodine concentration in product

C1 was determined to be 656 μg/ml based on the equation in the solasodine calibration curve (Figure 1).

Furthermore, analysis of solasodine content in product C2 done by HPLC was presented in Figure 5. The occurrence more than of 11 peaks at times having retention from 0.916 to 9.740 min were observed at the chromatogram. The HPLC chromatogram showed the width of area for the peak at 2.261 min identified as solasodine was 2,857,078). Based on the standard calibration curve of solasodine, solasodine content in product C2 was calculated to be 1,142 μg/ml from the basis of 10,000 μg/ml of the injected product C1 sample.

2. General description of goldfish infected by *Argulus* sp.

The fish used in this research was goldfish infected by the Argulus sp. parasite, which was observed in the abdominal, pectoral, and caudal fins, and redness in the infected organ (red arrows in Figure 6).

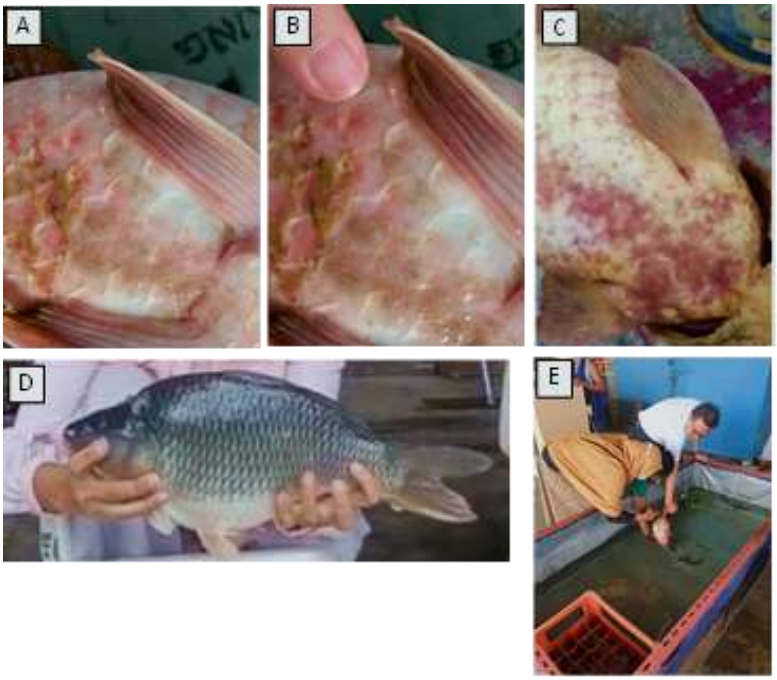


Figure 6. (A), (B), and (C) infected goldfish by Argulus sp. (D) experimental fish, (D) experimental pond for growing goldfish

Argulus sp. has a mouth with a stylet is to inject a digestive enzyme into the fish body and to absorb food that becomes liquid. This digestion process causes severe irritation and tissue damage in the gill tissue or infected skin (Noaman *et al*. 2010). Goldfish infected by Argulus sp. in the fish pond of Samarinda, East Kalimantan had hemorrhagic red spots, lesions, nectrotic wounds,

and ulceration (Figure 6); these findings agree with the results of Yıldız and Kumantas (Yildiz & Kumantas 2002).

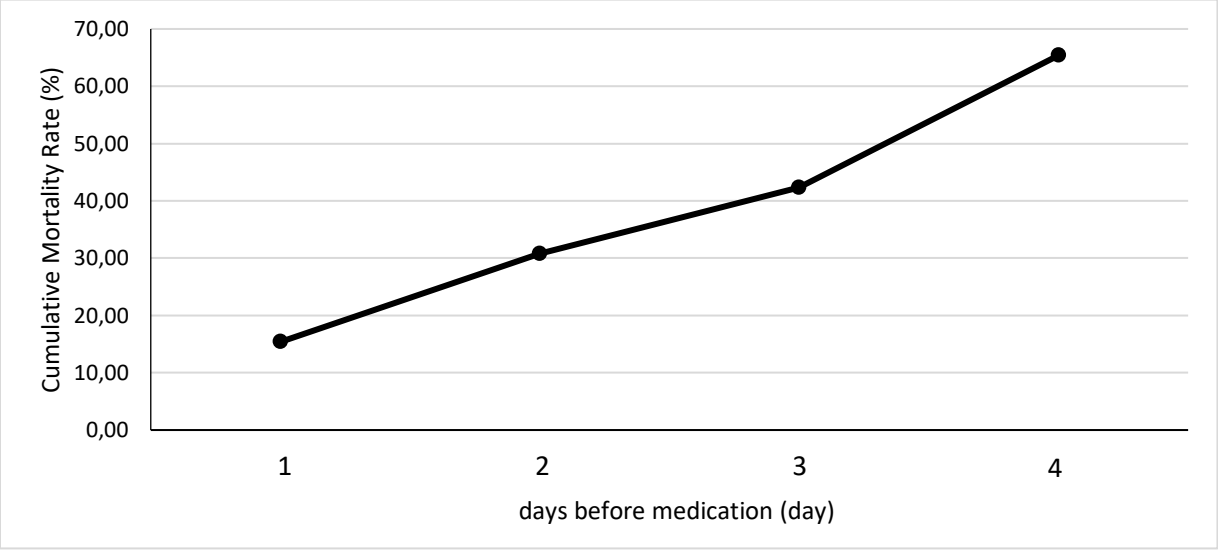


Figure 7. Cumulative mortality rate of goldfish in fish pond from 20th to 23rd of February 2021 before medication with extracts.

Fish death at the beginning of infection of *Argulus* sp. reached 15.38%, and the cumulative death kept increasing until 65.38% four days after infection (Figure 7). Hence, infection kept increasing, and dead fish had *Argulus* sticking to their body, with redness, wounds, and necrose (Figure 6).

3. *In vitro* testing of antiparasitic activities

The first stage of medication was performed by testing the effectiveness in SFE single extract and a concoction with BPE and ZZE against the *Argulus* sp. parasite *in vitro*. The results showed that single SFE (A2) in the *in vitro* test had the highest antiparasitic activities of up to 100% and killed *Argulus* sp. in 240 minutes (Figure 8).

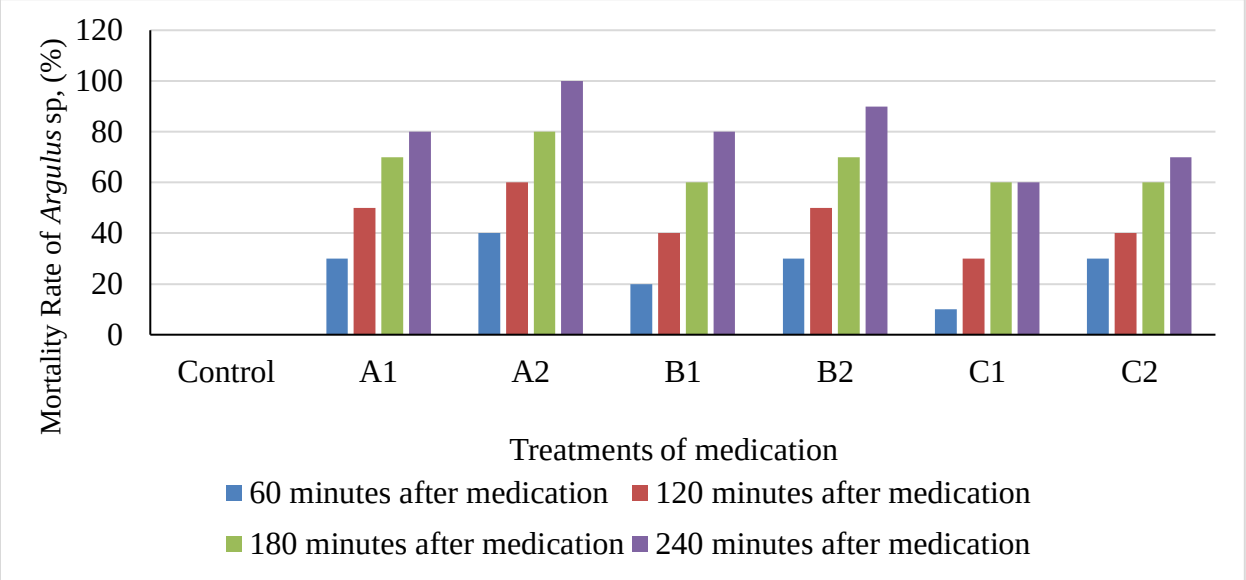


Figure 8. In vitro mortality of Argulus sp. treated with different concentrations of single and concoction extract of *S. ferox*, *B. pandurata*, and *Z. zerumbet* during 60, 120, 180, dan 240 minutes after medication (time observation).

The efficiency level of extract on the *Argulus* sp. parasite in vitro was approximately 70–100% in 240 minutes. This result shows the suitability of SFE (400 ppm) and ZZE (200 ppm) are the doses at 100 mL (A2) was capable of killing *Argulus* sp. (Table 1). Comprehensively, single (C1 and C2) and concoction extracts (A1, B1, and B2) had an efficiency higher than 50% in 180 and 240 minutes.

Table 1. In vitro study to estimate of antiparasitic efficacy (%) of *S. ferox*, *B. pandurata*, and *Z. zerumbet* extracts against *Argulus* sp.

Treatments	Observation time (minutes)			
	60	120	180	240
A1	30%	50%	70%	80%
A2	40%	60%	80%	100%
B1	20%	40%	60%	80%
B2	30%	50%	70%	90%
C1	10%	30%	60%	60%
C2	30%	40%	60%	70%

Some plant extracts were antibacterial and capable of killing *Argulus* sp. parasites. Ekanem *et al.* (2004) conducted *in vitro* testing on a rough extract of *Mucuna pruriens* and *Carica papaya* on a protozoa parasite. Extracts of Mucuna and Papaya oil had an effectiveness of

35% and 60%, respectively, after 3 hours, whereas 100% parasite death was observed at 6 hours when using 100 and 150 ppm. To determine the anthelmintic activities of a Henna herbal extract, Ayderke and Amedmado also showed the *in vitro* efficacy on an ectoparasite (Eguale *et al.* 2015). Similarly, Mehlhorn *et al*. (2011) evaluated the ovicidal effect in a product (Wash Away Louse), namely neem seed extract against nits on the body and head at different time intervals, and showed that a 5-minute incubation time was effective for preventing azny incubation. Meanwhile, approximately 4% and 76% of larva in the control without treatment hatched in sequential order.

4. Testing for antiparasitic efficiency of SFE, BPE, ZZE *in vivo* in goldfish

In vivo medication testing demonstrated that soaking with single extract and a concoction of SFE, BPE and ZZE extracts at different concentrations resulted in a significant decrease in goldfish infection. Table 2 shows the highest decrease of *Argulus* sp. infection in medication using SFE at 100 ppm of up to 81.33% (S.D. = 3.21%). The death level of *Argulus* sp. showed a significant difference ($P<0.05$) in all treatments. Based in Table 2, the treatment between B2 and C2 weren't show a significant difference, but when compared with the treatment K and A2 that showed a significant difference.

Table 2. (In vivo) concentration effect of extracts of BPE, SFE, and ZZE against Argulus sp. parasite in goldfish after 12 hours of treatment.

Treatment	Level of dead *Argulus* parasite (%)
K	0.00 ± 0.00^{a}
A2	81.33 ± 3.21^{b}
B2	75.67 ± 6.03^{c}
C2	71.00 ± 1.00^{c}

Some plant extracts have antibacterial and antiparasitic. In vivo testing showed that Azadirachtin resulted in a significant reduction in the Argulus burden of goldfish (Kumar *et al*. 2009). The in vivo use of two isolated compounds—arctigenin and arctiin—against gill parasites was found to be efficacious at 0.62 and 3.55 mgL-1, with 50% and 100% efficacies at 10.0 mg L-1, respectively (Wang *et al*. 2000). Similarly, in vivo testing of *Nerium oleander* extract in the destruction of *Argulus foliaceus* and *Lernaea cyprinacea* in ornamental fish (Sundus 2019).

Table 3 shows the effect of time interval in application of extracts from BPE, SFE, and ZZE against *Argulus* sp. in goldfish during 2, 4, 6, 8, 10, and 12 hours. The highest of average level of death *Argulus* sp. were 3.67% (B2 and C2 in 2

hours), 5.67% (A2 in 4 hours), 11.33% (B2 in 6 hours), 15.33% (A2 in 8 hours), 20.33% (A2 in 10 hours), 29.00% (A2 in 12 hours), respectively.

Table 3. Effect of time interval in application of extracts from BPE, SFE, and ZZE against Argulus sp. parasite in goldfish (in vivo test).

Treatment	Average level of dead *Argulus* (%)					
	2 hours	4 hours	6 hours	8 hours	10 hours	12 hours
K	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
A2	1.33±1.15	5.67±1.15	9.67±0.58	15.33±0.58	20.33±1.53	29.00±1.00
B2	3.67±1.15	4.67±0.58	11.33±1.53	14.33±1.15	18.67±1.53	23.00±1.00
C2	3.67±1.15	5.00±1.00	10.33±0.58	12.67±0.58	18.00±1.00	21.33±1.53

Figure 9 shows the mortality percentage of goldfish after medication with extracts. The percentage of mortality of goldfsh was decreased after soaking with the extract. On the first day, goldfish mortality was above 12%, but there was a significant decrease on the second day after treatment. No mortality was observed in the Goldfish on the fifth day after treatment.

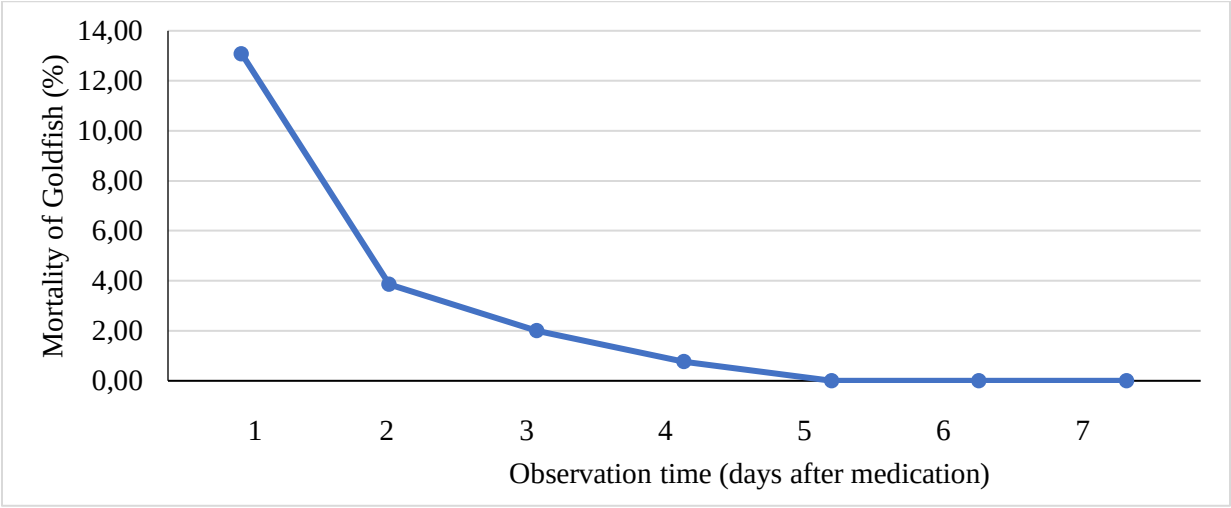


Figure 9. Mortality percentage of goldfish in fish pond from 24th of February 2021 to 2nd of March 2021, after medication with extracts.

Discussion

Our results demonstrate that for *in vitro* testing, the death rate of *Argulus* sp. was more rapid compared with the *in vivo* tests. This difference was caused by *Argulus* being vulnerable to the medication with the *in vitro* extract because it was removed from the host. Meanwhile, for *in vivo* medication, *Argulus* was protected by the host's scales and fins, in general agreement with *in vivo* and *in vitro* testing for all types of medication for fish (Natalie & Roy 2017). *In vitro* tests are feasible for assessing the intrinsic activities of a substance, but it is currently not impossible to replicate complex *in vivo* regulation (Natalie & Roy 2017).

Viewed from the exposure time of *Argulus* sp. with the extract, the amount of dead *Argulus* sp., as shown in Table 3, reached 29.00% ± 1.00. Regarding the *Argulus* infective dose in fish, 15–20 *Argulus* can cause moderate disease, leading to a low level of hemorrhage in some goldfish[22]. The capability of killing *Argulus* in extracts of SFE, BPE, and ZZE was excellent (71.00–80.33% in *in vivo* testing after 12 hours of medication). Testing with the Azadirachtin solution extract with the soaking method led to around 9.33–53.33% killing *in vivo* following 12 hours of medication.

The single *S. ferox* extract and concoction with BPE and ZZE had solasodine as a part of the steroidal glycoalkaloid. Solasodine can prevent food absorption and disturb growth in most insects (Krieg *et al.* 2016). Moretti *et al*. (1998) successfully isolated the natural steroid compound 3b-amino-22,26-epiminocholest-5-ene (sarachin) from an extract of Saraca punctate leaves, and this compound had killing activity against the malaria parasite—plasmodium falciparum—due to a series of amino steroids with side chains similar to sarachin. This explanation also applies to the antiparasitic activities of single SFE and the concoction with BPE and ZZE.

Solasodine is the most active derivative that might contribute to the antiparasitic activities. The advantage of using the hydrophobic steroid unit is its membrane permeability, opening a path for the biologically active hybrid molecule (Thongchai *et al.* 2011). On that basis, the synthesized steroid and concoction from the hydrophobic steroid group and hydrophobic group (ferrocenylmethylamino, N-alkil-pridinium groups) were responsible for the compounds' antimicrobial activities (Shafir & Van 2009). The antiparasitic activity of the higher concoction extract composition

demonstrates its increased antiparasitic capability. The solasodine concentrations in the single SFE and concoction with SFE and BPE as well as SFE and ZZE were 665 ppm, 485 ppm, and 295 ppm, respectively.

In sum, the *S. ferox* extract with solasodine, in the single extract and concoction, can be used for killing *Argulus* sp. parasites, whereas the concoction extract had a better antiparasitic effect.

Conclusion

In this research, a single extract of *S. ferox* and a concoction with SFE and BPE as well as SFE and ZZE were used to identify the antiparasitic effect against *Argulus* sp. infected in goldfish. The A2 (SFE 400 ppm and ZZE 200 ppm; 1:1) treatment is the best dose that can to kill *Argulus* sp. on goldfish based on in vitro and in vivo test.

Suggestion

Plant extracts concoctions have the best performance as antiparasitic in infected fish and this natural plant is effective in goldfish cultivation without side effects for curative medication and/or large-scale prevention and treatment.

Acknowledgments

All of the writers and researchers would like to express their gratitude to LPDP for providing the funds required to develop the natural fish drugs complete. In addition, they offer special thanks to students from the faculty of Fisheries and Marine Sciences, Mulawarman University, for their support and efforts in conducting this research.

References

Al Darwesh, A.A., Al Shabbani, M.A.A., Faris, B.H. 2014. Diagnostic and pathological study of Argulus japonicasin goldfish (*Carassius auratus*). G.J.B.B. 3 (4): 384-387.

Alom, M.Z., Yasmin, M.S., Rahman, M.A., Khan, S. 2019. Status, occurrence, intensity and impact of Argulosis in different brood stock ponds. MOJ Eco Environ Sci. 4(5): 225–229.

Asres, K., Bucar, F., Knauder, E., Yardley, V., Kendrick, H., Croft, S.L. 2001 In vitro antiprotozoal activity of extract and compounds from the stem bark of *Combretum molle*. Phytother Res. 15: 613–617.

Bibon, M.B. 2021. Antibacterial in-vitro evaluation of phenotypically screened solasodine from *Solanum nigrum* Linn. against enterohemorrhagic *Escherichia coli* (0157:H7). International Journal of Scientific Research in Research Paper. 7:43-48..

Buchmann, K., Jensen, P.B., Kruse, K.D. 2003. Effects of sodium percarbonate and garlic extract on *Ichthyophthirius multifiliis* theronts and tomocysts: in vitro experiments. N Am J Aquac, 65: 21–24.

Chang, W., Li Y., Zhang, M., Zheng, S., Li Y., Lou, H. 2017. Solasodine-3-O-β-d-glucopyranoside kills *Candida albicans* by disrupting the intracellular vacuole. Food and Chemical Toxicology. 106: 139-146.

Dewi, R.R., Siallagan,W., Suryanto, D. 2018. The efficacy of sodium chloride application in the control of fish lice (*Argulus* sp) infection on tilapia

(*Oreochromis niloticus*). Acta Aquatica: Aquatic Sciences Journal, 5(1): 4-7.

Eguale, T., Gebreyes, W.A., Asrat, D. 2015. Non-typhoidal Salmonella serotypes, antimicrobial resistance and co-infection with parasites among patients with diarrhea and other gastrointestinal complaints in Addis Ababa, Ethiopia. BMC Infect Dis 15: 497. https://doi.org/10.1186/s12879-015-1235-y

Ekanem, A.P., Obiekezie, A., Kloas, W., Knopf, K. 2004. Effects of crude extracts of *Mucuna pruriens* (Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite *Ichthyophthirius multifiliis*. Parasitol Res, 92:361–366

Hardi, E.H. 2021. *Boesenbergia pandurata* application in goldfish (*Cyprinus carpio*) feed to enhance fish growth, immunity, and resistance to bacterial infection. OSF. 2021. 10.17605/OSF.IO/827EN

Hardi, E. H. 2018. Borneo herbal plant extracts as a natural medication for prophylaxis and treatment of *Aeromonas hydrophila* and *Pseudomonas fluorescens* infection in tilapia (*Oreochromis niloticus*). F1000Res.;7. 30984371 10.12688/f1000research.16902.2 PMC6439779

Hardi, E. H. 2017. Immunomodulatory and antibacterial effects of *Boesenbergia pandurata*, *Solanum ferox*, and *Zingiber zerumbet* on tilapia, *Oreochromis niloticus*. AACL Bioflux. 10(2):182–190.

Hardi, E. H. 2018. Inhibition of fish bacteria pathogen in tilapia using a concoction three of Borneo plant extracts. In: IOP Conference Series. Earth and Environmental Science: IOP Publishing.

Hardi, E.H. 2019. Simultaneous Administration of *Boesenbergia pandurata* Extract and Vaccination to Stimulate Immune Response in Tilapia, *Oreochromis niloticus*. Pak J Biol Sci. 2019;22(9):419–426. 31930870 10.3923/pjbs.2019.419.426

Hardi, E.H. 2018. Uji in vitro Gabungan Ekstrak *Boesenbergia pandurata*, *Solanum ferox*, *Zingimber zerumbet* terhadap Bakteri Patogen pada Ikan Nila. Jurnal Veteriner. 19(1):35–44. 10.19087/jveteriner.2018.19.1.35

Hardi, E.H., N.R, Kusuma., I.W. 2019. Immunomodulatory effect and disease resistance from three Borneo plant extracts to Aeromonas hydrophila and Pseudomonas fluorescens in tilapia, Oreochromis niloticus. Aquacultura Indonesiana.,20(1):41–47. 10.21534/ai.v20i1.135

Hardi, E. H, Nugroho, R.A., Rostika, R., Mardliyaha., C.M., Sukarti, K., Rahayu, W., Supriansyah, A., Saptiani, G. 2022. Synbiotic application to enhance growth, immune system, and disease resistance toward bacterial infection in catfish (Clarias gariepinus), Aquaculture, 549, 737794, ISSN 0044-8486,

Hardi, E.H., Saptiani, G., Nugroho, R. A., Fadlul, R., Sulistyawati., W. Rahayu., A. Supriansyah, Irawan Wijaya Kusuma. 2021. Boesenbergia pandurata application in Goldfish (Cyprinus carpio) Feed to Enhancing Fish Growth, Immunity System, and Resistance to Bacterial Infection [version 2; peer review: 2 approved]. F1000Research 2021, 10:766 (https://doi.org/10.12688/f1000research.52889.2)

Hardi, E.H., Kusuma, I.W., Suwinarti, W., Agustina., Nugroho, R.A. 2016. Antibacterial activity of Boesenbergia pandurata, Zingiber zerumbet and Solanum ferox extracts against Aeromonas hydrophila and Pseudomonas sp. Nusantara Biosci, 8(1), 18–21. 10.13057/nusbiosci/n080105

Hardi, E.H., Saptiani, G., Kusuma, I.W., Suwinarti, W., Nugroho, R.A. 2016. Antibacterial activities of some Borneo plant extracts against pathogenic bacteria of Aeromonas hydrophila and Pseudomonas sp. AACL Bioflux, 2016a, 9(3):638–646.

Ijah, U.J, Oyebanji. F.O. 2003. Effects of tannins and polyphenols of some medical plants on bacterial agents of urinary tract infections. Global J.Pure Appl Sci 9:193–198

Jubair, N., Rajagopal, M., Chinnappan, S., Abdullah, N.B., Fatima, A. 2021. Review on the Antibacterial mechanism of plant-

derived compounds against multidrug-resistant bacteria (MDR). Evidence-Based Complementary and Alternative Medicine: 1-30.

Kabata, Z. 1985. Parasites and diseases of fish cultured in the tropics. Taylor & Francis, London and Philadelphia, PA.

Khandani, S.K., Mehrabani, M., Sharififar, F., Pardakhty, A., Pournamdari, M., Pakravanan, M. 2019. Development and validation of an RP-HPLC method for determination of solasodine, a steroidal alkaloid. J.Young Pharm 11: 21-25.

Kipp, R.M, Larson, J., Fusaro, A., Makled, T.H, Benson, A.J. 2014. *Argulus japonicus*. USGS Nonindigenous Aquatic Species Database. [Cited 13 November 2013] Available at: http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=166.

Natalie, S. and Roy, P.E.Y. 2017. Argulus (Fish Louse) Infections in Fish. U.S. Department of Agriculture, UF/IFAS Extension Service, University of Florida, IFAS, Florida A & M University Cooperative Extension Program, and Boards of County Commissioners Cooperating. Nick T. Place, dean for UF/IFAS Extension. Pp 4

Krieg, R., E. Jortzik., Alice-Anne Goetz3, Ste´phanie Blandin3, Sergio Wittlin4,5, Mourad Elhabiri6, Mahsa Rahbari2, Selbi Nuryyeva6,7, Kerstin Voigt8, Hans-Martin Dahse8, Axel Brakhage8, Svenja Beckmann9, Thomas Quack9, Christoph G. Grevelding9, Anthony B. Pinkerton10,11, Bruno Scho¨necker12, Jeremy Burrows13, Elisabeth Davioud-Charvet6, Stefan Rahlfs2 & Katja Becker. 2016. Arylmethylamino steroids as antiparasitic agents. Nature Communication. 8:14478 | DOI: 10.1038/ncomms14478 | www.nature.com/naturecommunications

Kumar P, Sharma B, Bakshi N. 2009. Biological activity of alkaloids from *Solanum dulcamara* L. Nat Prod Res 23: 719-23.

Lamarre, E., and P.A. Cochran. 1992. Lack of host species selection by the exotic parasitic crustacean Argulus japonicus. Journal of Freshwater Ecology 7(1):77-80.

Madsen HCK, Buchmann K, Mellergaard S (2000) Treatment of trichodiniasis in eel (*Anguilla anguilla*) regarded in recirculation systems in Denmark: alternatives to formaldehyde. Aquaculture 186:221–231

Mandalakis,M.; Anastasiou, T.I.; Martou, N.; Keisaris, S.; Greveniotis, V.; Katharios, P.; Lazari, D.; Krigas, N.; Antonopoulou, E. Antibacterial Effects of Essential Oils of Seven Medicinal-Aromatic Plants against the Fish *Pathogen Aeromonas veronii* bv. sobria: To Blend or Not to Blend?. Molecules 2021, 26, 2731. https://doi.org/10.3390/ molecules26092731.

Mehlhorn, H., Abdel-Ghaffar, F., Al-Rasheid, K.A., Schmidt, J., Semmler, M.. Ovicidal. 2011. Effects of a neem seed extract preparation on eggs of body and head lice. Parasitol Res. 109(5):1299-302. doi: 10.1007/s00436-011-2374-8. Epub 2011 Apr 12. PMID: 21484346.

Moretti, C. 1998. A novel antiprotozoal aminosteroid from Saracha punctata. J. Nat. Prod. 61, 1390–1393.

Noaman, V., Chelongar, Y., Shahmoradi, A. 2010. The first record of *Argulus foliacesus* (Crustacea: Branchiura) infestation on lionhead goldfish (*Carassius auratus*) in Iran. J. Parasitol 5:71–76.

Noga. 2000. Fish Diseases ; Diagnosis and Treatment. Iowa State Press. A Blackwell. United State pp. 85-90.

Pandey, A.K., Nagpure, N.S., Trivedi, S.P., Kumar, R., Kushwaha, B. 2011. Profenofos Induced DNA Damage In Freshwater Fish, *Channa punctatus* (Bloch) Using Alkaline Single Cell Gel Electrophoresis. Mutation Research 726:209-214. www.elsevier.com/locate/gentox/ Tanggal Akses 2 Mei 2012.

Satrija, F., Retnani, E.B., Ridwan, Y., Tiuria, R. 2001. Potential use of herbal anthelmintics as alternative antiparasitic drugs for small holder farms in developing countries. In: Livestock community and environment. Proceedings of the 10th Conference of the

Association of Institutions for Tropical Veterinary Medicine, Denmark

Shafir, A., Van, A. J. 2009. Laying, development, and hatching of eggs of the fish ectoparasite *Argulus japonicus* (Crustacea, Branchiura). J Zool 210:401–413.

Shen, K.H., Hung, J.H., Chang, C.W., Weng, Y.T., Wu, M.J., Chen, S.P. 2017. Solasodine inhibits invasion of human lung cancer cell through downregulation of miR-21 and MMPs expression. Chemico-Biological Interactions 268: 129-135.

Singh, P., Singh, P., Singh, M.P. 2019. Quantitative estimation of steroidal drug solasodine in wild species of Solanum by reversedphase HPLC method. Journal of Pharmacognosy and Phytochemistry 8: 920-926

Steckler, N., Yanong, R.P.E. 2012. Argulus (fish louse) infections in fish. UF/IFAS Fisheries and Aquatic Sciences. [Cited 10 November 2013]. Available at http://edis.ifas.ufl.edu/fa184

Sundus, W.A. 2019. Study of the effect of Nerium oleander extract in the destruction of Argulus foliaceus and Lernaea cyprinacea in ornamental fish International journal of Research in Pharmaceutical Sciences. 10(4): 3073-3077.

Thongchai, W., Liawruangrath, B., Liawluangrath, S., Saysin, S. 2011. High Performance Liquid Chromatographic determination of solasodine in solanum species. Asian Journal of Chemistry 23: 2407-2410.

Tona, L., Kambu, K., Ngimbi, N., Climanga, K., Vlietinck, A.J. 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J Ethnopharmacol 61:57–65

Wang, L.D., Guo, D.A., Yuan, L., He. Q.H., Hu, Y.Q., Tu, P.F. 2000. Antifungal effect of three natural products on the genetic substance of *Saccharomyces cerevisiae* GL7 and *Prototheca wickerhamii*. Yao Xue Xue Bao. 35(11): 860-863

Willcox, M.L., Bodeker, G. 2000. Plant-based malaria control: research initiative on traditional antimalaria methods. Parasitol Today 16:220–221

Xu, X.H., Zhang, L.L, Wu, G.S., Chen, X, Li. T., Chen, X., Wang, Y.T., Lu, J.J. 2017. Solasodine induces apoptosis, affects autophagy, and attenuates metastasis in ovarian cancer cells. Planta Med 83: 254-260.

Yıldız, K.K.A., Kumantas, A. 2002. Argulus foliaceus infection in a goldfish (Carassius auratus). Isr J Vet Med 57:118–120.