

Antiparasitic Activity of Single *Solanum ferox* Extract and Concoction with *Zingiber zerumbet* and *Boesenbergia pandurata* Extracts to Control *Argulus* sp. on Goldfish (*Cyprinus carpio*)

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Abstract: *Argulus* is an ectoparasite that frequently infects goldfish, with severe effects and a high death rate. The use of plant extracts has been widely reported to have antiparasitic activities, including one of the solasodine compounds in sour eggplant extracts. This research evaluated the antiparasitic activities of Compositions A (*Solanum ferox* and *Zingiber zerumbet* extract at 400 and 200 ppm, respectively), B (SFE and *Boesenbergia pandurata* at 900 ppm), and C (single *Solanum ferox* at 400 ppm). The research stages comprised 1) isolation and analysis of solasodine content in sour eggplant extracts and its extract composition, 2) *in vitro* antiparasitic activities on *Argulus* sp. at doses of 50 and 100 ppm with a testing time of 60–240 minutes, and 3) *in vivo* antiparasitic activities on *Argulus* sp. on goldfish at a dose of 100 ppm and observation for 2–12 hours of medication. The results showed that sour eggplant extracts had a solasodine concentration of 7.151 mg/L, whereas the derivative compositions A, B, and C were 656, 485, and 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* medication 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: *Argulus* sp., antiparasitic, natural product, fish diseases, solasodine, *Solanum ferox*.

1. 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 [1]. 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 [2].

According to Steckler and Yanong [3], *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 [4-6]. 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 [3].

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 [7]. Furthermore, according to Lamarre and Cochran [8], 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 chloride⁹ and trichlorfon at 0.25 ppm [7]. 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 [10]. 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 [11-15]. Extract of garlic (*Allium sativum*) is capable of killing trichodinids [16], theronts, and tomonets from the *Ichthyophthirius multifiliis* parasite [17]. Ekanem et al. [18] 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 [19,20], antibacterial [21-24,47,48], anticancer [25,26,45,46], and antiparasitic potencies as well as several other important activities [27].

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* [28-36,47,48]. 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.

2. Results

2.1. 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 $y = 0.0004x + 0.2702$ (Figure 1. fr) and correlation coefficient 0.9724. HPLC analysis showed a peak of solasodine retained at 2.379 minutes.

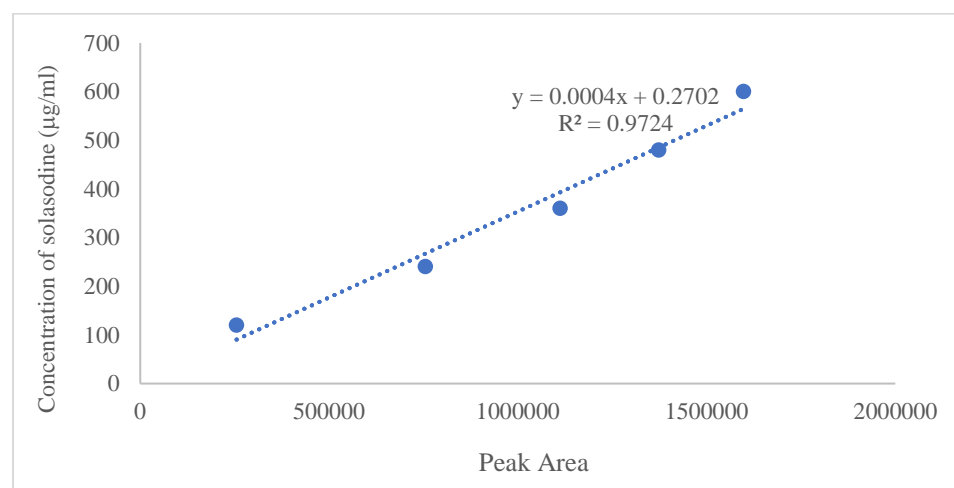


Figure 1. Standard Calibration curve of \underline{s} ranging from 120 to 600 $\mu\text{g/ml}$ in mobile phase.

B. Analysis of solasodine content in sour eggplant 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.

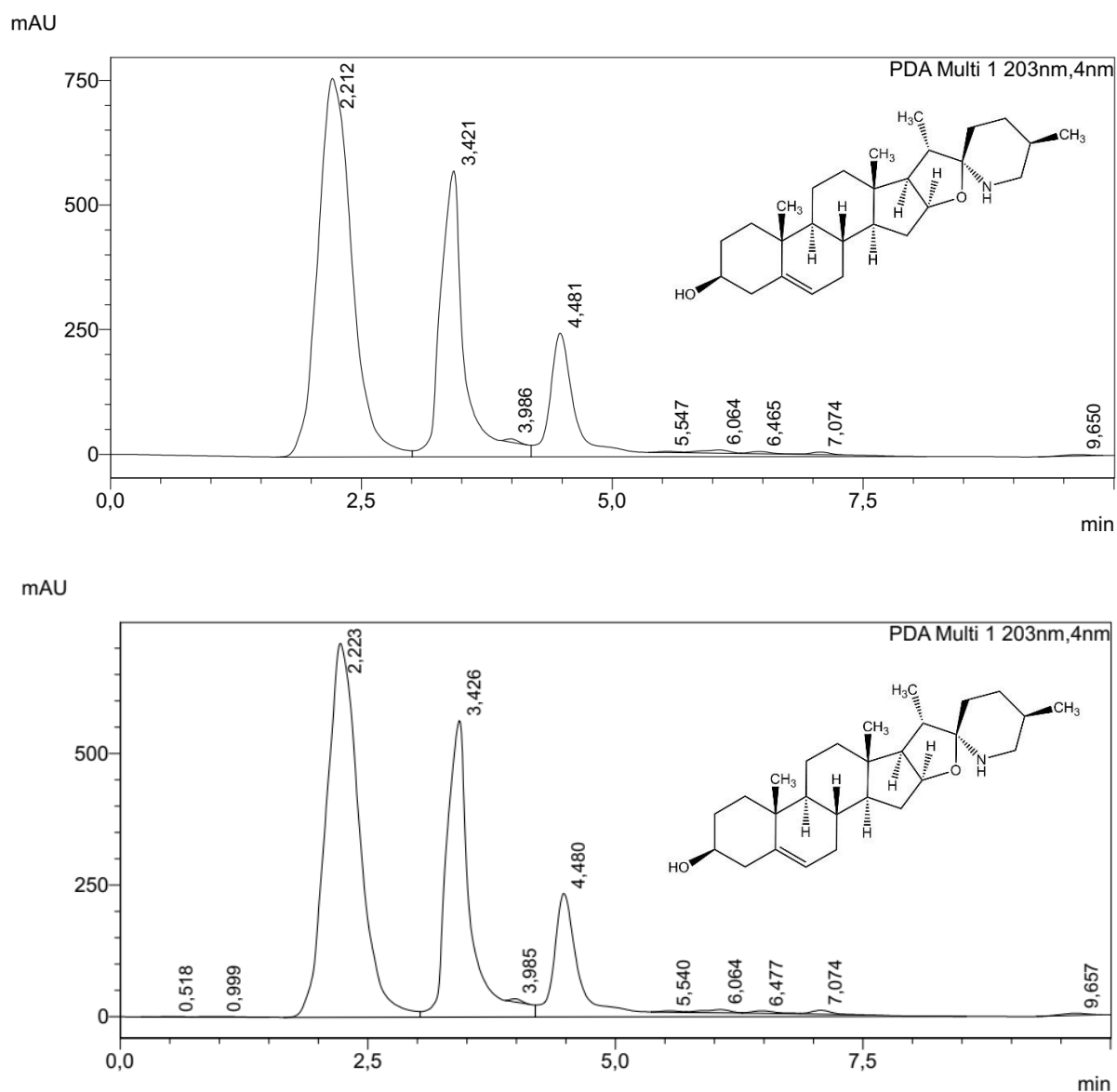


Figure 2. HPLC chromatogram of SFE 1 (above, t_R 2.212 min) and SFE 2 (below, t_R 2.223 min) showing the occurrence of solasodine in sour eggplant extract.

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 $\mu\text{g}/\text{ml}$ from the basis of 10,000 $\mu\text{g}/\text{ml}$ 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 $\mu\text{g/ml}$ based on the 10,000 $\mu\text{g/ml}$ 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.

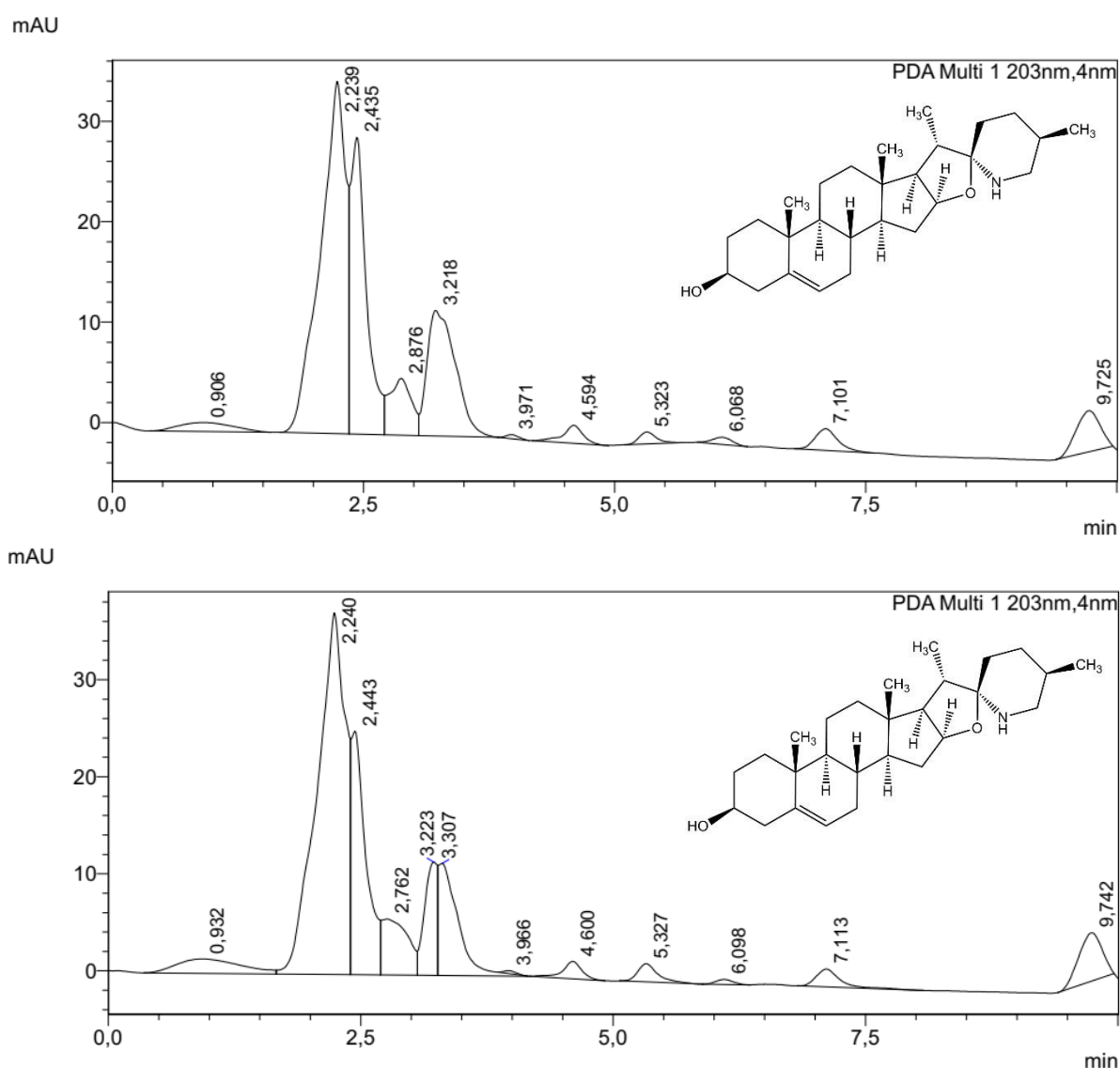


Figure 2. HPLC chromatogram of products A1 (above, t_R 2.239 min) and A2 (below, t_R 2.240 min) showing the occurrence of solasodine in sour eggplant extract

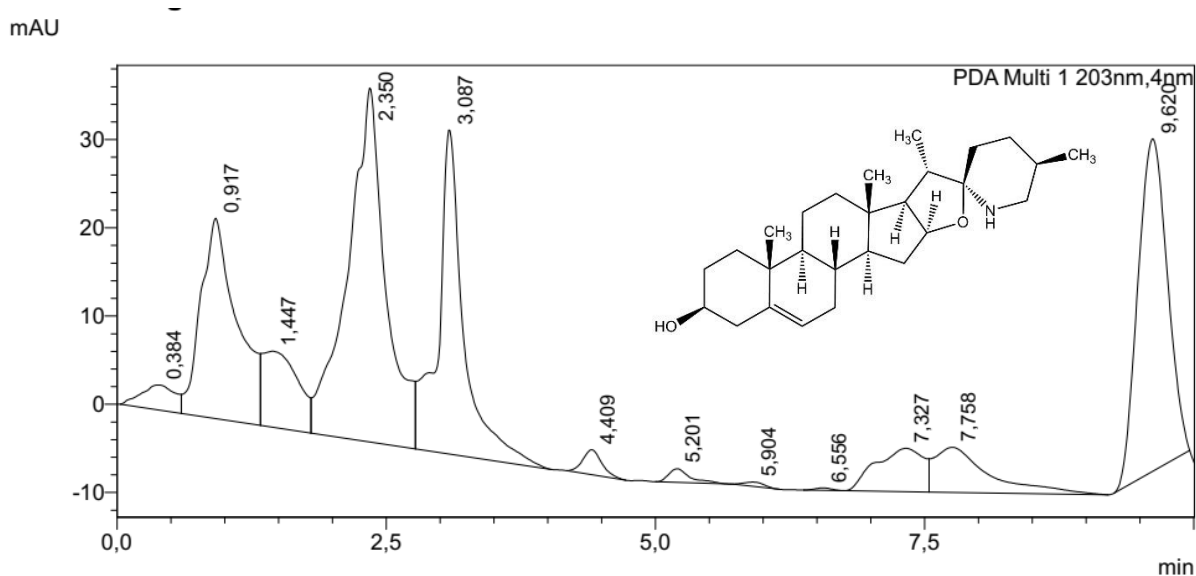
The HPLC chromatogram showed a peak 2.239 min that is identified as solasodine with the width of area was 622,008. Based on the solasodine calibration curve, the solasodine content in the product A1 was calculated as much as 248 $\mu\text{g/ml}$.

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. The solasodine concentration in product A2 based on the equation in the solasodine calibration curve (Figure 1) was calculated to be 295 $\mu\text{g/ml}$.

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 Fig 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.



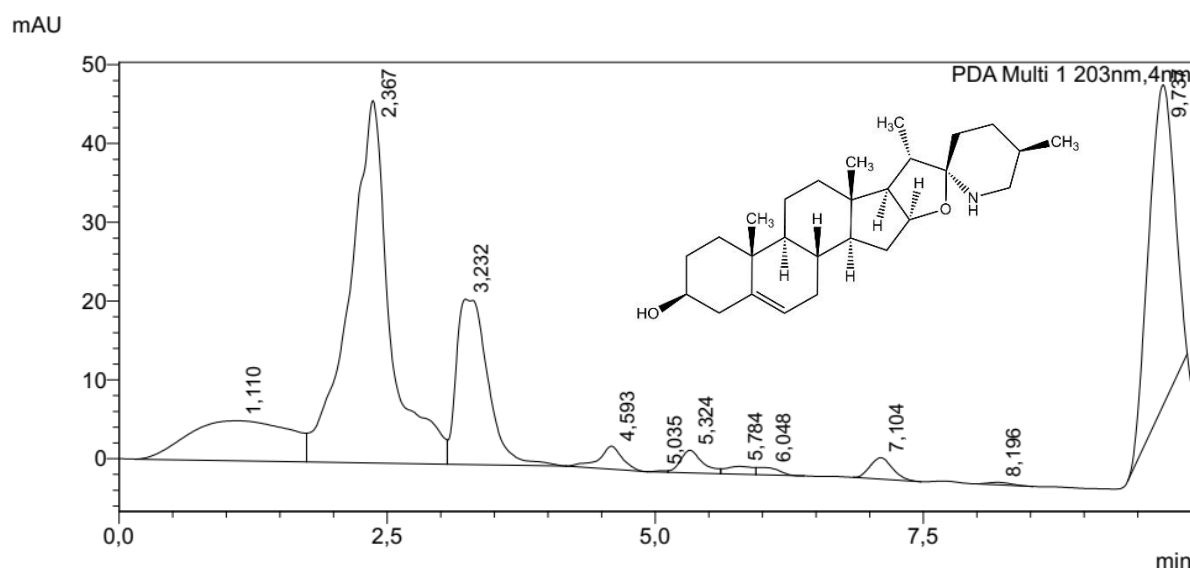


Figure 4. HPLC chromatogram of products B1 (above, t_R 2.350 min) and B2 (below, t_R 2.367 min) showing the occurrence of solasodine in sour eggplant products.

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 $\mu\text{g/ml}$ based on the 10,000 $\mu\text{g/ml}$ of injected volume of the product.

Solasodine content in product B2 was analyzed by HPLC as shown in Figure 4. Eleven peaks were presented with the retention times of 1.110 - 9.737 min. Solasodine occurrence 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 presented in Fig 5. More than 12 peakswith the retention from 0.975 to 9.740 min were detected at the chromatogram.

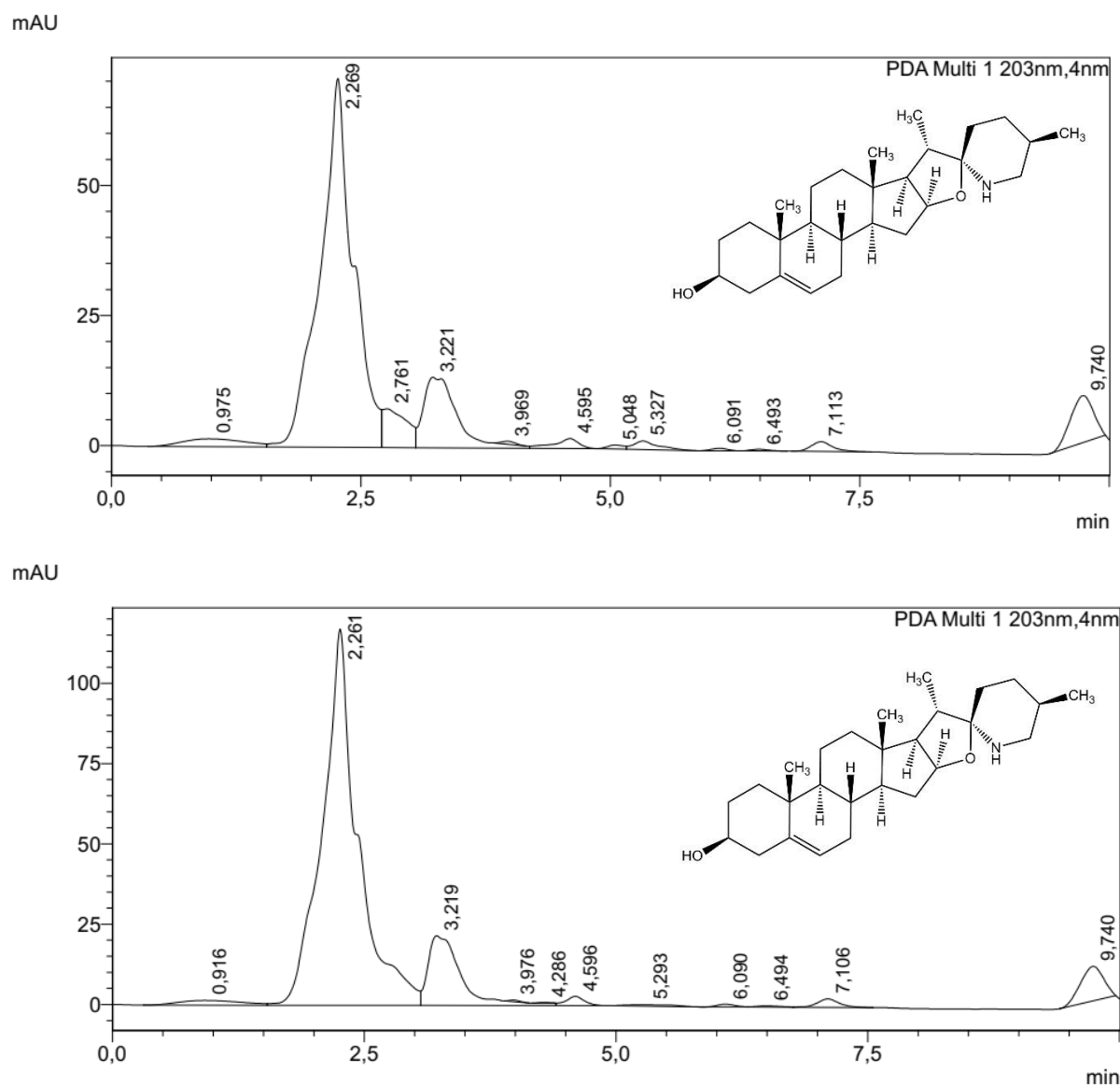


Figure 5. HPLC chromatogram of products C1 (above, t_R 2.269 min) and C2 (below, t_R 2.261 min) showing the occurrence of solasodine in sour eggplant products

The presence of solasodine in product C1 was indicated by the peak at 2.269 min as presented in Fig 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 $\mu\text{g}/\text{ml}$ 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 $\mu\text{g/ml}$ from the basis of 10,000 $\mu\text{g/ml}$ of the injected product C1 sample.

2.2. 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 (Figure 10). *Argulus* sp. has a mouth with a stylet, which is used 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 [7]. Goldfish infected by *Argulus* sp. in the fish pond of Samarinda, East Kalimantan had hemorrhagic red spots, lesions, necrotic wounds, and ulceration (Figure 10); these findings agree with the results of Yıldız and Kumantas [37].

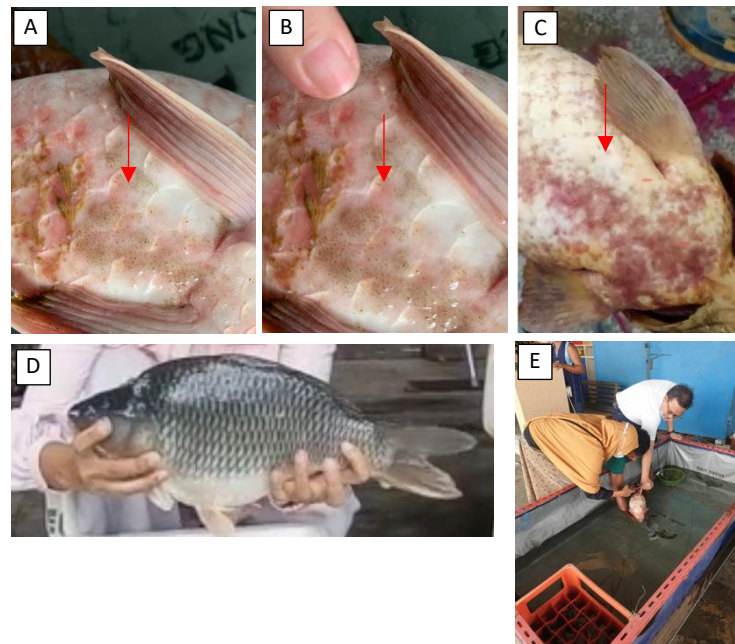


Figure 10. (A), (B), and (C) goldfish infected by *Argulus* sp. (D) goldfish used for experiments, (E) pond for growing goldfish.



Figure 11. Cumulative mortality rate of goldfish in fish pond from 20th to 23rd of February 2021, before medication with extract.

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 11). Hence, infection kept increasing, and dead fish had *Argulus* sticking to their body, with redness, wounds, and necrose (Figure 10).

2.3. *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 12).

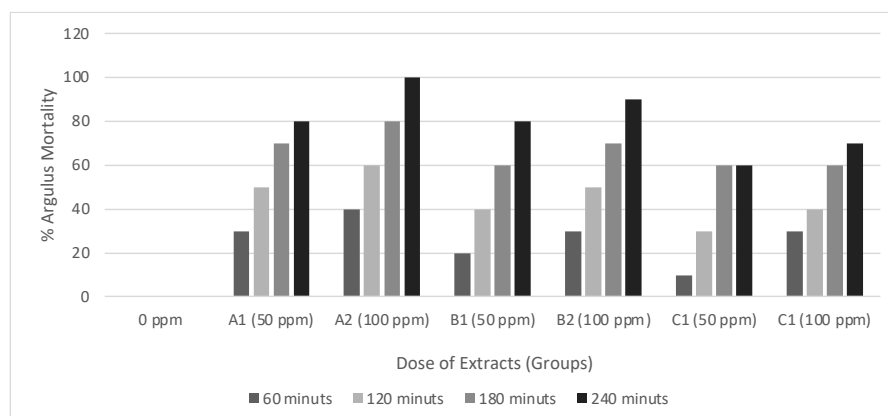


Figure 12. *In vitro* mortality of *Argulus* sp. treated with different concentrations of single and concoction extract of *S. ferox*, *B. pandurata*, and *Z. zerumbet*.

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 using SFE (at 400 ppm), whereas

the dose at 100 ppm was capable of killing *Argulus* sp. at a rate of 100% (Table 1). Comprehensively, single and concoction extracts had an efficiency higher than 50% in 180 and 240 minutes.

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

Treatment	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. [18] 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 [38]. Similarly, Mehlhorn et al. [39] 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 any incubation. Meanwhile, approximately 4% and 76% of larva in the control without treatment hatched in sequential order.

2.4. 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%) and a significant difference from the control and other compositions ($P < 0.05$).

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

Treatment	Level of dead <i>Argulus</i> parasite (%)
K	0 ±0.00 ^a
A2	81.33 ± 3.21 ^b
B2	75.67 ± 6.03 ^c
C2	71.00 ± 1.00 ^c

Some plant extracts were antibacterial and antiparasitic. *In vivo* testing showed that Azadirachtin resulted in a significant reduction in the *Argulus* burden of goldfish [22]. 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 [19]. Similarly, *in vivo* testing of Nerium oleander extract in the destruction of *Argulus foliaceus* and *Lernaea cyprinacea* in ornamental fish [40].

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

Treatment	Average level of dead <i>Argulus</i> (%)					
	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

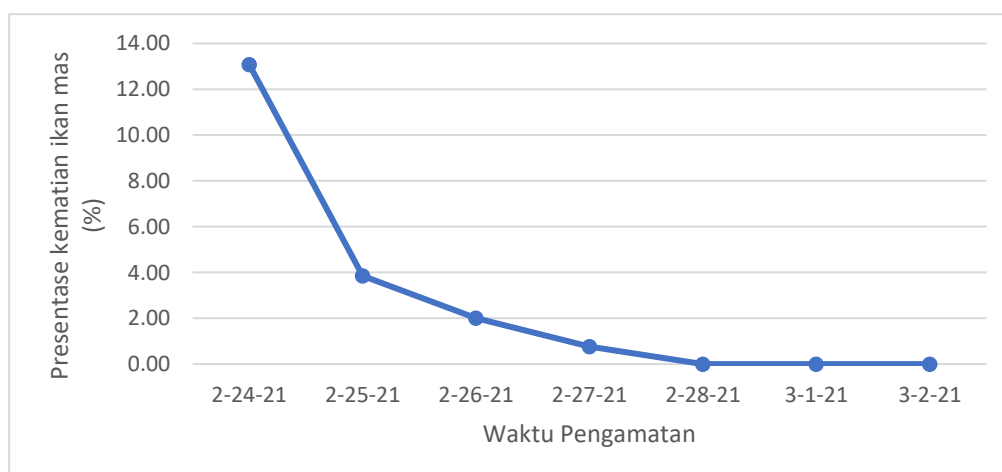


Figure 13. Mortality percentage of goldfish in fish pond from 24th of February 2021 to 2nd of March 2021, before medication with extracts of SFE, BPE, and ZZE.

3. 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 [41]. *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 [41].

Viewed from the exposure time of *Argulus* sp. with the extract, the amount of dead *Argulus* sp., as shown in Table 3, reached $29.00\% \pm 1.00$. Regarding the *Argulus* infective dose in fish, 15–20 *Argulus* can cause moderate disease, leading to a low level of hemorrhage in some goldfish²². 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 [27]. Moretti et al. [42] successfully isolated the natural steroid compound 3b-amino-22,26-epiminocholest-5-ene (sarachin) from an extract of *Saraca punctata* 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 [44]. On that basis, the synthesized steroid and concoction from the hydrophobic steroid group and hydrophobic group (ferrocenylmethylamino, N-alkyl-pridinium groups) [43] were responsible for the compounds' antimicrobial activities. 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.

4. Material and Methods

4.1. Experimental Fish

Fish used in this research were goldfish (*Cyprinus carpio*) with a weight of 4–5 kg 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*.

4.2. 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. [28,29], 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 [28-36].

The *Argulus* medication experiment in goldfish used three different extract compositions, namely:

Composition A : *Solanum ferox* (SFE 400 ppm) and *Zingiber zerumbet* (ZZE 200 ppm) extracts, ratio 1:1.

Composition B : SFE (400 ppm) and *Boesenbergia pandurata* (BPE 900 ppm) extracts with ratio 1:1.

Composition C : single SFE (400 ppm) extract.

4.3. 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 °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 ZZS), 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.

4.4. *In vitro* testing of parasitic activities from plant extracts

In vitro analyses were conducted following the procedure of Ekanem et al. [18]. *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 ppm.

A2, *Argulus* was soaked with composition A at 100 ppm.

B1, *Argulus* was soaked with composition B at 50 ppm.

B2, *Argulus* was soaked with composition B at 100 ppm.

C1, *Argulus* was soaked with composition C at 50 ppm.

C2, *Argulus* was soaked with composition C at 100 ppm.

The antiparasitic efficacy of each group was calculated using the equation of Wang et al. [19]:

$$AE = (B - T) / B \times (100\%)$$

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.

4.5. *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 ppm had better antiparasitic activities, compared with 50 ppm; hence, the *in vivo* tests used four treatments, as follows:

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

A2 treatment, fish soaked with composition 1 at 100 ppm.

B2 treatment, fish soaked with composition 2 at 100 ppm.

C2 treatment, fish soaked with composition 3 at 100 ppm.

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 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.

4.6. Statistical analysis

The collected data were analyzed using the statistical package SPSS version 16.

5. 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. infecting goldfish. The inhibitive traits in the test results were affected by content and synergy, observed in most of the concoction examined, in which the SFE and concoction with BPE and ZZE. In practice, 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.

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Conceptualization, E.H.H ; R.A. N; Methodology, E. H. H, R.A.N, Y.F, I.W.K, and G.S; Formal Analysis, E.H.H, and R.A.N; Investigation, E.H.H, M.A, A.A, A.A, B.M, T. W, N.N, and A.L.; Resources, E.H.H and R.A.N; Writing—Original Draft Preparation, E.H.H and R.A.N; Writing—Review & Editing, E.H.H, I. W. K, and R.A.N; Supervision, E.H.H and R.A.N; Project Administration A.A and Y.F. All authors have read and agreed to the published version of the manuscript.

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