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RESEARCH ARTICLE

The Antiparasitic Potential of *Senna alata* Leaves Extracts and Fractions Against Marine Parasitic Leech *Zeylanicobdella arugamensis*

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Abstract Aquaculture has been a rapidly growing industry in Malaysia, contributing to the country's gross domestic product and enhancing food security and employment opportunities. Among the marine species cultivated in Malaysia are sea bass, snapper, and grouper. Unfortunately, the marine parasitic leech *Zeylanicobdella arugamensis* is posing a major threat to a wide variety of cultured marine fish species, including grouper. Various veterinary drugs are used to control parasitic leeches, which are toxic to fish and humans. So, the development of natural treatments from plants is vital. In response to this, *Senna alata*, also known as the Ringworm bush, was selected. This study aims to evaluate the antiparasitic activity of *S. alata* methanol extracts, its fractions and water extract against *Z. arugamensis*. The adult and mature leeches were divided into control and treatment groups. The methanol extract, its fractions (including hexane, chloroform, ethyl acetate, and butanol), and water extracts of *S. alata* were obtained. Various concentrations (100, 50, 25 and 12.5 mg/ml) of the extracts and fractions were prepared in 5% DMSO and concurrent notation of physico-chemical parameters. Upon exposure, the behaviour and mortality time of the parasitic leeches were recorded. The exposure to the extracts and fractions induced behavioural changes in the parasitic leeches, characterised by aggressive and abnormal swimming patterns coupled with an inability to attach their suction to the surface. Ultimately, complete mortality of parasitic leeches was obtained across extracts and fractions. Overall, the aqueous extract proved to be the most effective, as it displayed the shortest mortality time recorded among the extracts and fractions (2.38±0.15 to 33.08±3.52 minutes). Consequently, the study highlights the antiparasitic potential of the extracts and fractions of *S. alata* against parasitic leech *Z. arugamensis* infestation.

Keywords: *Senna alata*, leech, fractions, solvent extract, aquaculture. *Zeylanicobdella arugamensis*.

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Introduction

Aquaculture has been one of Malaysia's fastest-growing industries since the 1920s, contributing 8.9% of the whole nation's gross domestic product [1]. It shows great promise as a means of guaranteeing food security and promoting long-term development. With breakthroughs in genetic breeding programmes and cutting-edge culture system technologies, the sector has made significant progress. Notably, Malaysia has excellent management techniques and access to high-quality aquaculture feed, as underlined by Fathi *et al.* (2018)[2]. Malaysia's aquaculture industry is seeing extraordinary expansion. According to the Promotion of Sustainable Aquaculture in Malaysia 2021 study, the sector has seen widespread participation from diverse regions, indicating consistent development efforts. In 1992, Malaysia provided only 7% of the national fish production[3]. However, by 2003, this percentage

had nearly quadrupled to around 13%, indicating a healthy growth trend that is expected to continue. Aquaculture production has increased dramatically over the years, rising from less than 80,000 metric tons in 1992 to more than 427,000 metric tons in 2017, with a market value of MYR 3 billion. This expansion not only demonstrates Malaysia's dedication to aquaculture but also highlights the country's ability to meet the growing demand for seafood, which is being driven by an expanding population. Aside from its critical function in maintaining food security, aquaculture contributes significantly to job creation, commercial opportunities, and investment chances in Malaysia. The sector employed over 18,000 aquafarmers nationwide in 2017, managing a total farm area of more than 34,000 hectares. Such statistics highlight the socioeconomic importance of aquaculture, establishing it as a significant driver of Malaysia's agricultural landscape and economic prosperity [2,3]

Malaysia's aquaculture industry predominantly focuses on brackish water, but freshwater and marine environments also host aquaculture activities. Among the variety of marine species cultivated in Malaysia are sea bass, snapper and grouper [1,2,4,5]. Grouper possesses high market demand locally and internationally, particularly in upscale restaurants during the festive seasons [6]. Aquaculture of grouper can help reduce the strain on overfished populations, especially in the Asia-Pacific region [7]. Indonesia, along with Taiwan and mainland China, dominated worldwide grouper aquaculture production, accounting for 11%, 17%, and 65%, respectively [8]. However, one of the difficulties associated with the rearing of groupers is the infestation by the marine parasitic leech *Zeylanicobdella arugamensis*, [9–11].

The *Z. arugamensis* species was first discovered in Sri Lanka, but, because of changes in the global ecology, it is now widespread throughout the Indian Ocean. It was initially believed to have been discovered in Malaysia, coming from the seahorse *Hippocampus kuda* and an unidentified cell species. It has been observed that *Z. arugamensis* is one of the most harmful ectoparasites that affects a wide variety of fish species and poses a significant risk to the aquaculture business. Within three days of being infested, it will cause mortality in the host, followed by a secondary infection with dangerous bacteria such as *Vibrio agnolyticus* [12]. Physically *Z. arugamensis* exhibits an elongated cylindrical body shape with anterior and posterior sections. The anterior suction is larger than the posterior one. The anterior section is distinguished by an oval-shaped sucker with a diameter of 0.3–0.4 mm and is surrounded by a flower-shaped cuticle with little white cilia measuring 0.01 mm in length. Meanwhile, there are five pairs of testes and a proboscis on the ventral side [13]. The marine parasitic leech attaches to the eyes, mouth, fins, gills and body surface of the host (Figure 1)[9–11]

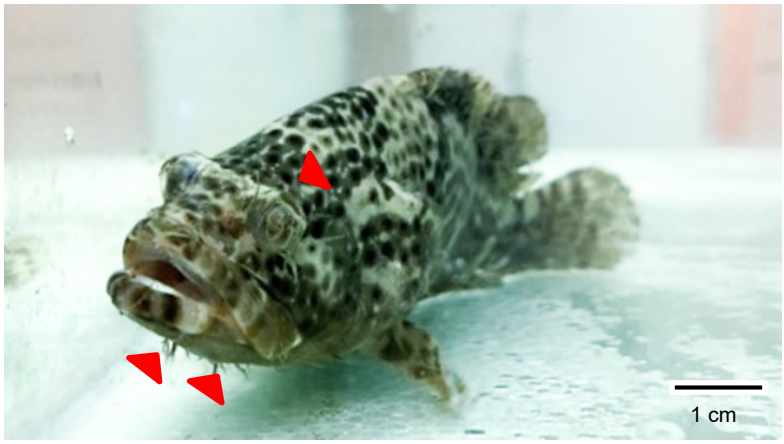

Figure 1. Marine parasitic leech-infesting hybrid grouper (*Epinephelus fuscoguttatus x E. lanceolatus)* The parasitic leeches are attached to the fins, gills, mouth area, and body surface of the host

In aquaculture, veterinary medications are used for the prevention or treatment of diseases. However, studies have found chemical parasiticide side effects, including buildup in fish tissues and negative effects on the fish's indigenous microflora. Aside from that, the buildup of antiparasitic and chemical residues in water has influenced the ecosystem, particularly in aquaculture in open waters where medications are difficult to control. These chemical residues in the environment may have deadly or sublethal impacts on nontarget creatures. For example, when pesticides such as Neguvon and Nuvon were employed to control sea lice (*Lepeophtheirus salmonis)* in salmon net-pen farming in Norway, various crustaceans around the farms were affected [14]. A potential solution is the application of natural products derived from plant extracts. This approach reduces reliance on toxic chemicals,

promoting the production of organic food [15,16]. Herbal medications are emerging as an alternate approach to aquaculture disease. Management. This herbal medicine offered its effectiveness as a growth promoter, preventive and therapeutic agent, and immune system modulator. The efficacy of the herbal plant emerges from the secondary metabolites, including saponins, alkaloids, tannins, phenolics, polyphenols, lignins, glycosides, polypeptides, etc [17,18]. In the current study, we focus on *Senna alata*, also known as Candle bush or Ringworm bush (Figure 2). This herbal plant belongs to the family Leguminosae. Indigenous to the Philippines, Sri Lanka, Malaysia, etc. The extracts and isolated pure compounds of the plant have been found to have a variety of bioactivities such as antioxidant, antimicrobial, antiviral, antimalarial, antiparasitic, etc. This could be due to the presence of different metabolites, including alkaloids, flavonoids, phenolics, tannins, terpenes, anthraquinones, steroids, quinones, saponins, reducing sugars, and volatile oils [19].

Figure 2. *Senna alata* or Ringworm bush (Leguminosae) leaves are paripinnate, asymmetrically triangular, and persistent, with 25-75 cm long rachis and oblong, asymmetrically rounded leaflets

Materials and Methods

Sample Collection

The fresh leaves of the plant were collected in September 2023 from Kampung Piasau, Membakut, Sabah (5° 28' 41.88"N, 115° 46' 5.28"E). The necessary permits for sample collection in Sabah were obtained from the Sabah Biodiversity Centre (SaBC) (License No.: JKM/MBS.1000-2/13 JLD. 2 (32). The species was identified by expert Johnny Gisil at the Institute of Tropical and Biological Conservation, University Malaysia Sabah, and specimen voucher no (BORH 5582) was obtained.

Extraction

The leaves of the plant were rinsed with tap water to remove any parasites, fungus, and decayed materials and dried at room temperature. The dried leaves were ground into a fine powder using a heavy-duty grinder. About 60 g of dry powdered leaves were dissolved in 100% HPLC-grade methanol with a ratio of 1:5 ratio (w/v) powder methanol. After mixing, the solution was put inside the incubator shaker for 48 hours at room temperature. Mesh and Whatman filter paper were used to filter the extract to remove suspended particles. The methanol residue was removed using a vacuum rotary evaporator. The samples were kept at -80 ^{0}C for 24 h and then lyophilized using a freeze drier. The freeze-dried samples were then stored in the freezer for further analysis [20].

Fractionation

The dried methanol crude extract was further fractionated with water and various solvents (ratio 1:10 w/v) such as n-hexane, chloroform, ethyl acetate, and butanol in a separatory funnel with increasing order of polarity. The resulting mixture was shaken and allowed to settle. The solvent residue was removed using a vacuum rotary evaporator. The samples were kept at -80 ^{0}C for 24 hours and then lyophilized using a freeze dryer. The freeze-dried samples were then stored in the freezer for further analysis [21].

Aqueous Extraction

Using a stirring hot plate, about 60 g of dry plant powder was boiled for 10 minutes with distilled water at a 1:10 ratio (w/v). The decoctions were removed and left to cool at room temperature for one hour. The extracts were then filtered using a sieve to remove coarse residues before being filtered again with the Whatman No. 1 paper. After 24 hours at -80°C, the filtrate was lyophilized with a freeze drier.

Extraction Yield

The extraction yield which involves the weight of extract obtained and the weight of dried powder was calculated by using Equation 1 below

$$\frac{Weight\ of\ the\ extract\ obtained}{Weight\ of\ dried\ powder} \times 100\% \quad (1)$$

Marine Parasitic Leech Collection

The marine parasite leeches, Z. arugamensis, were collected from Borneo Marine Research Institute, Universiti Malaysia Sabah aquaculture facilities. The parasitic leech-infested hybrid grouper *(Epinephelus fuscoguttatus x Epinephelus lanceolatus)* weighs about 250 to 350 g with a diameter of 15–25 cm. The fish was taken out of the cage, put into a small seawater tank, and the leeches were removed by hand (Figure 1). The gathered leeches were transferred to another container.

Antiparasitic Bioassay

In the current study, a total of 405 healthy and mature parasitic leeches (*Z. arugamensis*) were selected. The leeches were divided into 27 groups, with 5 leeches per group. Group 1 served as a negative control and received seawater treatment only. Groups 2 and 3 served as positive controls and received 0.25% v/v formalin and freshwater treatment, respectively. Groups 4 to 27 were exposed to 4 different concentrations (100, 50, 25, and 12.5 mg/ml) of methanol extract, its fractions (hexane, chloroform, ethyl acetate, and butanol), and the aqueous extract prepared. The highest concentration of the methanol extract and fraction was prepared by dissolving them in a solution containing 5% DMSO (dimethyl sulfoxide) prepared in seawater. Meanwhile, the aqueous extract was dissolved solely in seawater. Following exposure, the control and treated parasitic leeches were observed for more than 3 hours in a petri dish [20]. The experiment was done in triplicate to ensure the accuracy and reliability of the results.

The Behaviour of Parasitic Leeches and Mortality Time

The behaviour of the parasitic leech was monitored in the control and extract-treated groups. Which include swimming patterns, body movement, and suction attachment to the surface. In addition, the mortality time of parasitic leeches was monitored using a stopwatch. Once the parasitic leeches ceased movement, they were physically poked to confirm their lack of responsiveness. if there was no movement observed, they were transferred back to the seawater and monitored for some time to confirm their deaths. Leeches that displayed no movement during the observation period were considered dead [22].

Physico-Chemical Parameters

The water quality parameters of the control and treated solutions were assessed. The temperature and pH levels were determined using a pH meter, while the dissolved oxygen (DO) content was measured using a D.O. meter (Eutech DO 2700 Meter, Thermo Fisher Scientific, America). Additionally, the salinity level was determined using a refractometer (Handheld Aquarium ATC Refractometer, RCYAGO, China).

Statistical Analysis

The IBM SPSS Statistics 29.0 software was used to analyse the data. The Kruskal-Wallis's test and Dunnett T3 multiple comparison tests were used to determine significant differences between groups exposed to various concentrations of extracts and fractions. The data were shown as mean ± standard deviation. A p-value < 0.05 indicated a significant result.

Results and Discussion

This investigation into evaluating the antiparasitic potential of the leaves of *S. alata* methanol extract, along with its fractions and water extract, involved multiple analyses. Initially, the percentage yield of the extracts and fractions of *S. alata* was calculated. Later, the physio-chemical parameters of the control solutions and different concentrations of the extracts and fractions were determined. Further, to determine the antiparasitic potential of *S. alata*, the behaviour and mortality time of the marine parasitic leeches exposed to the control solutions

and different concentrations of the extracts and fractions were observed and recorded. The detailed results of these analyses are presented in subsequent sections.

Table 1 presents the percentage yields of the methanol extract, its fractions (hexane, chloroform, ethyl acetate and butanol) and the aqueous extract obtained from *S. alata.* Between extracts, the methanol extract exhibits the highest yield percentage, compared to the aqueous extract. Among the fractions, butanol exhibited the highest percentage yield followed by chloroform, ethyl acetate and hexane.

Table 1. Percentage yields of methanol extract, its fractions and aqueous extract of *S. alata*

No.	***Senna alata* extracts/fractions**	**Percentage yield (%)**
1	Methanol extract	5.74 ± 0.31
2	Hexane fraction	0.43 ± 0.01
3	Chloroform fraction	1.72 ± 0.02
4	Ethyl acetate fraction	0.80 ± 0.01
5	Butanol fraction	2.23 ± 0.01
6	Aqueous extract	3.78 ± 1.41

Table 2 illustrates the water quality parameters observed in the control and treatment solutions, including temperature, pH, dissolved oxygen, and salinity. Overall minimum discrepancies were noted in these parameters except for pH, which displayed fluctuations compared to the control and treatment solution. Notably, the pH tended towards the neutral values with reduction of the concentration of treatment solutions.

Table 2. Water quality parameters of control and treatment solutions

No	**Group**	**Concentration (mg/ml)**	**Temperature (° C)**	**pH**	**Dissolved oxygen (mg/l)**	**Salinity (ppt)**
1.	Negative control (Seawater)		24.9	7.37	7.56	30.8
2.	Positive control (0.2% Formalin)	0.2%	24.8	6.94	7.63	30.6
3.	Positive control (Freshwater)		24.8	7.46	8.03	0.5
4.	Methanol	100	24.1	4.00	6.00	28.0
5.		50	24	4.10	6.34	27.3
6.		25	24	4.41	6.46	27.2
7.		12.5	24.3	5.65	6.21	27.4
8.	Hexane	100	24.7	4.82	6.16	27.4
9.		50	24.9	5.45	6.44	27.0
10.		25	25.2	6.54	6.29	27.2
11.		12.5	24.8	7.21	6.01	28.0
12.	Chloroform	100	23.7	4.26	6.48	28.3
13.		50	23.8	4.94	6.44	27.8
14.		25	24.2	6.43	6.00	27.5
15.		12.5	23.7	6.86	6.19	28.0
16.	Ethyl acetate	100	23.7	4.07	6.46	28.8
17.		50	24	4.69	6.62	28.4
18.		25	23.9	5.12	6.00	27.8
19.		12.5	24.3	6.46	6.16	27.9
20.	Butanol	100	23.5	4.00	6.67	27.4
21.		50	23.6	4.10	6.44	28.8
22.		25	23.6	5.01	6.22	28.6
23.		12.5	23.8	6.42	6.09	27.9
24.	Aqueous	100	24	5.04	6.43	27.6
25.		50	23.9	5.22	6.21	27.0
26.		25	23.9	5.41	6.01	27.6
27.		12.5	23.9	5.87	6.14	27.1

Upon exposure to normal seawater, the parasite leech exhibited unaltered behaviour. However, when subjected to a 0.2% formalin treatment and fresh water, the leech became notably active and aggressive, displaying abnormal movement. They were not able to attach their suction to the bottom or wall of the petri dish. In the *S. alata* treated groups, exposure to 100 mg/ml and 50 mg/ml concentrations of the extracts and fractions induced high activity and aggression in the leeches and prevented them from attaching their suction to the base of the petri dish. Conversely, when subjected to lower concentrations of 25 mg/ml or 12 mg/ml, the leeches displayed less aggression as compared to high concentrations, and they were able to attach their suction to the base of the petri dish before eventual mortality. Upon death, they slowly detached their suction from the base of the petri dish. Figure 3 (A-D) shows the parasite leech group treated with *S. alata* aqueous extract. Once the movement ceased, the leeches were transferred back to the seawater to confirm their deaths, but no further movement was noticed.

Figure 3. *S. alata* aqueous extract treated parasite leech groups. A = 100 mg/ml, B = 50 mg/ml, C= 25 mg/ml and D = 12.5 mg/ml

Table 3 shows the recorded mortality time of parasite leeches. Complete mortality was noticed in a dose-dependent manner across the methanol extract, its fractions, and the aqueous extract. Notably, the aqueous extract at 100 and 25 mg/ml concentrations exhibited rapid parasitic leech mortality compared to the methanol extract and its fractions, except the ethyl acetate fraction at 50 mg/ml, which outperformed the aqueous extract at the same dosage.

Table 3. Mortality time of leech after exposure to various concentrations of extracts, fractions, and controls

No	Group	Concentration (mg/ml)	Mortality time (minutes) Mean± S.D
1.	Negative control (Seawater)		> 180 ±0.0
2.	Positive control (0.2% Formalin)	0.2%	7.04 ± 0.58[a]
3.	Positive control (Freshwater)		121.34 ± 21.67 [ab]
4.	Methanol	100	8.23±3.06 [ac]
5.		50	20.96±10.61 [ac]
6.		25	28.17±9.72 [abc]
7.		12.5	95.00±15.62 [abc]
8.	Hexane	100	12.31±4.26 [ac]
9.		50	69.79±29.14 [abce]
10.		25	181.52±11.52 [abcf]
11.		12.5	193.90±15.58 [abcg]
12.	Chloroform	100	12.53±2.04 [abc]
13.		50	13.90±3.17 [abc]
14.		25	66.83±23.62 [abcf]
15.		12.5	146.28±38.54 [abg]
16.	Ethyl acetate	100	6.83±1.28 [ac]
17.		50	8.21±2.90 [abc]
18.		25	47.19±15.52 [abc]
19.		12.5	85.65±2.36 [abc]
20.	Butanol	100	9.08±1.86 [ac]
21.		50	15.45±6.20 [ac]
22.		25	22.52±5.00 [abc]
23.		12.5	84.67±1.83 [abc]
24.	Aqueous	100	2.38±0.15 [abcd]
25.		50	14.31±3.45 [abc]
26.		25	17.58±2.33 [abc]
27.		12.5	33.08±3.52 [abc]

Each value represents the mean±S.D. of 5 parasitic leeches per group.
[a] Significance at $p<0.05$ compared with the normal control: seawater.
[b] Significance at $p<0.05$ compared with the positive control: formalin (0.25%)
[c] Significance at $p<0.05$ compared with the positive control: freshwater.
[d] Significance at $p<0.05$ compared with the methanol extract for 100 mg/ml.
[e] Significance at $p<0.05$ compared with the methanol extract for 50 mg/ml.
[f] Significance at $p<0.05$ compared with the methanol extract for 25 mg/ml.
[g] Significance at $p<0.05$ compared with the methanol extract for 12.5 mg/ml.

According to the overall results the methanol extract exhibits the highest yield percentage compared to the aqueous extract. This difference may arise from the solvent's varying polarity and affinities for extracting certain chemicals, with methanol demonstrating higher efficacy than aqueous in extracting specific compounds from plant material [23]. Among the fractions, butanol exhibited the highest percentage yield compared to the hexane, chloroform, and ethyl acetate fractions. The difference can be attributed to butanol's selective solubility as a polar aprotic solvent capable of dissolving a wide range of molecules, both polar and nonpolar. This characteristic allows butanol to extract a wide range of chemicals, potentially contributing to a higher overall yield when compared to other solvents [24].

The water quality parameters in the normal control and extracts and fractions solutions displayed variations in the pH. The fluctuations in pH values could be attributed to the presence of bioactive compounds with acidic properties in *S. alata* extract [19]. The results are in comparison with previous data [11].

The antiparasitic potential of *S. alata* was evaluated by exposing parasitic leech *Z. arugamensis* to methanol extract, its four different fractions (hexane, chloroform, ethyl acetate, and butanol) and aqueous extract at various concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml) to determine their efficacy. Each concentration exhibited different average leech mortality times across the extracts and fraction groups. Notably, at 100 mg/ml concentration, the aqueous extract demonstrated the shortest time for leech mortality (2.38 ± 0.15 minutes), outperforming other extract and fraction groups. Similarly, at 50 mg/ml concentration, the ethyl acetate fraction showed the shortest time for leech mortality (8.23±3.06 minutes). The trend continued with the 25 mg/ml concentration, where the aqueous extract recorded the shortest time (17.58 ± 2.33 minutes). At 12.5 mg/ml concentration, the aqueous extract again exhibited the shortest time (33.08 ± 3.52 minutes). Other studies also showed that the plant extracts are effective against parasitic leech species, such as *Melastoma malabathricum* (Melastomataceae, Straits Rhododendron), *Tetracera indica* (Dilleniaceae, Sandpaper vine), *Piper betle* (Piperaceae, betel), and *Etlingera coccinea* (Zingiberaceae, perennial ginger), were tested for antiparasitic activity against *Z. arugamensis*. These plant extracts displayed antileech properties in less than 5 minutes at a concentration of 0.25 g/ml. The leeches were immediately paralyzed, with their bodies hardening, shrinking, and turning a darker colour after being exposed to these extracts [25]. The aqueous extract of *Azadirachta indica* (Meliaceae, Neem) demonstrated potent antiparasitic activity against *Z. arugamensis*, achieving 100% mortality at concentrations of 100 mg/ml in average times of 6.45 minutes [22]. The aqueous extract of *Nephrolepis biserrata* (Nephrolepidaceae, Sword fern) at a concentration of 100 mg/ml resulted in parasitic leech mortality of around 11 minutes [26]. Similarly, the aqueous-ethanol extracts of *Zingiber officinale* (Zingiberaceae, ginger), *Ocimum basilicum* (Lamiaceae, basil), and *Castela texana* (Simaroubaceae, bitter chaparro) have demonstrated toxic effects against a marine parasitic leech *Neobenedenia* species (Monogenea: Caspsalidae) afflicting orange-spotted grouper (*Epinephelus coioides*) [17]. Thus, the plant extract can be used as an alternative against parasitic leech infestations. Plant-based remedies are both biodegradable and environmentally sustainable. Aquaculture techniques can be linked with sustainability goals by using plant-derived biochemicals, reducing environmental effects. In West Java, Indonesia, a large number of farmers used traditional medicinal plants in their aquaculture practices[16].

The extract of *S. alata* has been reported to have various metabolites that exhibit antiparasitic properties. Among these metabolites, kaempferol, emodin, luteolin anthraquinones etc. have been reported to have antiparasitic effects [19,27–30]. We believe the antiparasitic potential of *S. alata* extracts and fractions might be achieved due to the presence of these antiparasitic metabolites. However, further research is needed to isolate and identify metabolites present in the extracts and fractions of *S. alata* and evaluate their efficacy against the parasitic leech, *Z. arugamensis.* In addition, the extract of *S. alata* has also been reported to have antiparasitic properties against *Plasmodium berghei.* which further shows the antiparasitic nature of *S. alata* extract [31].

Conclusions

In the current study, the antiparasitic potential of *Senna alata* (known as 'Ringworm bush) is evaluated. The dry powder was extracted with both methanol and water, with the methanol extract further fractionated using hexane, chloroform, ethyl acetate, and butanol. The methanol extract shows a high extraction yield compared to aqueous extract. Further, *Zeylanicobdella arugamensis*, a marine parasitic leech, was exposed to varying concentrations of *S. alata* extracts and fractions ranging from 100 to 12 mg/ml. The findings revealed that all extracts and fractions exhibited significant antiparasitic potential, causing 100% mortality among the leeches. Overall, the aqueous extract proved the most

effective, as it displayed the shortest mortality time recorded among the extracts and fractions. This antiparasitic potential might be due to the presence of relevant chemical constituents within *S. alata*. Consequently, the study highlights the antiparasitic potential of the extracts and fractions of *S. alata* against parasitic leech *Z. arugamensis* infestation. The study holds potential benefits for fish farmers, particularly those engaged in grouper and sea bass aquaculture, for controlling parasite infestations. However, further research is essential to isolate the potential antiparasitic bioactive compounds found in *S. alata* and test them against the parasitic leech *Z. arugamensis.*

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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References

[1] Kurniawan, S. B., Ahmad, A., Rahim, N. F. M., Said, N. S. M., Alnawajha, M. M., Imron, M. F., Abdullah, S. R. S., Othman, A. R., Ismail, N. 'Izzati, Hasan, H. A. (2021). Aquaculture in Malaysia: Water-related environmental challenges and opportunities for cleaner production. *Environmental Technology & Innovation, 24*, 101913. https://doi.org/10.1016/j.eti.2021.101913

[2] Fathi, S., Harun, A. N., Rambat, S., & Tukiran, N. A. (2018). Current issues in aquaculture: Lessons from Malaysia. *Advanced Science Letters, 24*, 503–505. https://doi.org/10.1166/asl.2018.12051

[3] Jumatli, A., & Ismail, M. S. (2021). Promotion of sustainable aquaculture in Malaysia. In *Proceedings of the Promotion of Sustainable Aquaculture, Aquatic Animal Health, and Resource Enhancement in Southeast Asia: Proceedings of the International Workshop on the Promotion of Sustainable Aquaculture, Aquatic Animal Health, and Resource Enhancement in Southeast Asia* (pp. 31–40). Aquaculture Department, Southeast Asian Fisheries Development Center.

[4] FAO Food and Aquaculture Organization. (2024). Malaysia national aquaculture sector overview. Available online: https://www.fao.org/fishery/en/countrysector/naso_malaysia (accessed March 14, 2024).

[5] Aripin, A., Coglan, L., Pascoe, S., & Hoang, V. N. (2020). Productive efficiency and capacity utilization of sea bass grow-out culture in Peninsular Malaysia. *Aquaculture Economics & Management, 24*, 102–121. https://doi.org/10.1080/13657305.2019.1661045

[6] Rudd, M. A. (2004). The effects of seafood import tariffs on market demand for Nassau grouper in the Turks and Caicos Islands. *Proceedings of the Gulf and Caribbean Fisheries Institute, 55*, 178–190.

[7] Das, S. K., Xiang, T. W., Noor, N. M., De, M., Mazumder, S. K., & Goutham-Bharathi, M. P. (2021). Temperature physiology in grouper (Epinephelinae: Serranidae) aquaculture: A brief review. *Aquaculture Reports, 20*, 100682. https://doi.org/10.1016/j.aqrep.2021.100682

[8] Herdiana, Y., Wiryawan, B., Wisudo, S. H., Tweedley, J. R., Yulianto, I., Retnoningtyas, H., & Loneragan, N. R. (2024). Untangling the complexity of small-scale fisheries: Building an understanding of grouper-snapper fisheries dynamics in Saleh Bay, West Nusa Tenggara, Indonesia. *Fishes, 9*(2). https://doi.org/10.3390/fishes9010002

[9] Kua, B. C., Azmi, M. A., & Hamid, N. K. A. (2010). Life cycle of the marine leech (Zeylanicobdella arugamensis) isolated from sea bass (Lates calcarifer) under laboratory conditions. *Aquaculture, 302*, 153–157. https://doi.org/10.1016/j.aquaculture.2010.02.029

[10] Kua, B. C., Choong, F. C., & Leaw, Y. Y. (2014). Effect of salinity and temperature on marine leech, Zeylanicobdella arugamensis (De Silva) under laboratory conditions. *Journal of Fish Diseases, 37*, 201–207. https://doi.org/10.1111/jfd.12087

[11] Shah, M. D., Venmathi Maran, B. A., Haron, F. K., Ransangan, J., Ching, F. F., Shaleh, S. R. M., Shapawi, R., Yong, Y. S., & Ohtsuka, S. (2020). Antiparasitic potential of Nephrolepis biserrata methanol extract against the parasitic leech Zeylanicobdella arugamensis (Hirudinea) and LC-QTOF analysis. *Scientific Reports, 10*. https://doi.org/10.1038/s41598-020-79094-4

[12] Ravi, R., & Shariman Yahaya, Z. (2017). Zeylanicobdella arugamensis, the marine leech from cultured crimson snapper (Lutjanus erythropterus), Jerejak Island, Penang, Malaysia. *Asian Pacific Journal of Tropical Biomedicine, 7*, 473–477. https://doi.org/10.1016/j.apjtb.2017.01.018

[13] Diputra, W. A., & Bendryman, S. S. (2022). Identification of morphology of Zeylanicobdella arugamensis in Tiger Grouper (Epinephelus fuscoguttatus) using scanning electron microscope method in Lampung Bay, Indonesia. *Journal of Aquaculture Science, 7*, 49–56. https://doi.org/10.31093/joas.v7i2.255

[14] Wunderlich, A. C., Zica, É. de O. P., Ayres, V. F. dos S., Guimarães, A. C., & Takeara, R. (2017). Plant-derived compounds as an alternative treatment against parasites in fish farming: A review. In *Natural Remedies in the Fight Against Parasites*. InTech.

[15] Rico, A., Satapornvanit, K., Haque, M. M., Min, J., Nguyen, P. T., Telfer, T. C., & van den Brink, P. J. (2012). Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: A critical review. *Reviews in Aquaculture, 4*, 75–93. https://doi.org/10.1111/j.1753-5131.2012.01062.x

[16] Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture, 433*, 50–61. https://doi.org/10.1016/j.aquaculture.2014.05.048

[17] Trasviña-Moreno, A. G., Ascencio, F., Angulo, C., Hutson, K. S., Avilés-Quevedo, A., Inohuye-Rivera, R. B., & Pérez-Urbiola, J. C. (2019). Plant extracts as a natural treatment against the fish ectoparasite Neobenedenia sp. (Monogenea: Capsalidae). *Journal of Helminthology, 93*, 57–65. https://doi.org/10.1017/S0022149X17001122

[18] Shah, M. D., Tani, K., Yong, Y. S., Ching, F. F., Shaleh, S. R. M., Vairappan, C. S., & Venmathi Maran, B. A. (2021). Antiparasitic potential of chromatographic fractions of Nephrolepis biserrata and liquid chromatography-quadrupole time-of-flight-mass spectrometry analysis. *Molecules, 26*, 499. https://doi.org/10.3390/molecules26020499

[19] Oladeji, O. S., Adelowo, F. E., Oluyori, A. P., & Bankole, D. T. (2020). Ethnobotanical description and biological activities of Senna alata. *Evidence-Based Complementary and Alternative Medicine, 2020*, 1–12. https://doi.org/10.1155/2020/2580259

[20] Shah, M. D., Venmathi Maran, B. A., Tan, J. K., Yong, Y. S., Fui Fui, C., Shaleh, S. R. M., & Shapawi, R. (2021). The anti-leech potential of the solvent extract of Bornean neem leaves and ultra-high performance liquid chromatography-high-resolution mass spectrometry profiling. *Journal of King Saud University - Science, 33*, 101541. https://doi.org/10.1016/j.jksus.2021.101541

[21] Osadebe, P. O., Okoye, F. B. C., Uzor, P. F., Nnamani, N. R., Adiele, I. E., & Obiano, N. C. (2012). Phytochemical analysis, hepatoprotective and antioxidant activity of Alchornea cordifolia methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. *Asian Pacific Journal of Tropical Medicine, 5*, 289–293. https://doi.org/10.1016/S1995-7645(12)60041-8

[22] Venmathi Maran, B. A., Josmeh, D., Tan, J. K., Yong, Y. S., & Shah, M. D. (2021). Efficacy of the aqueous extract of Azadirachta indica against the marine parasitic leech and its phytochemical profiling. *Molecules, 26*, 1908. https://doi.org/10.3390/molecules26071908

[23] Abbas, A., Naqvi, S. A. R., Rasool, M. H., Noureen, A., Mubarik, M. S., & Tareen, R. B. (2021). Phytochemical analysis, antioxidant and antimicrobial screening of Seriphidium oliverianum plant extracts. *Dose-Response, 19*, 1–9. https://doi.org/10.1177/15593258211004739

[24] Okolie, A. C., Kale, O. E., & Osilesi, O. (2019). Chemoprotective effects of butanol fraction of Buchholzia coriacea (Capparidaceae) against type 2 diabetes and oxidative stress in male Wistar rats. *Bioscience Reports, 39*, 1–23. https://doi.org/10.1042/BSR20170665

[25] Wan Norhana, M. N., Kua, B. C., & Liyana, R. (2021). Evaluation of selected plant extracts for in vitro anti-marine leech (Zeylanicobdella arugamensis) activity. *Tropical Biomedicine, 38*, 122–129. https://doi.org/10.47665/tb.38.1.021

[26] Venmathi Maran, B. A., Palaniveloo, K., Mahendran, T., Chellappan, D. K., Tan, J. K., Yong, Y. S., Lal, M. T. M., Joning, E. J., Chong, W. S., Babich, O., & et al. (2023). Antimicrobial potential of aqueous extract of giant sword fern and ultra-high-performance liquid chromatography–high-resolution mass spectrometry analysis. *Molecules, 28*, 6075. https://doi.org/10.3390/molecules28166075

[27] Periferakis, A., Periferakis, A.-T., Troumpata, L., Periferakis, K., Scheau, A.-E., Savulescu-Fiedler, I., Caruntu, A., Badarau, I. A., Caruntu, C., & Scheau, C. (2023). Kaempferol: A review of current evidence of its antiviral potential. *International Journal of Molecular Sciences, 24*, 16299. https://doi.org/10.3390/ijms242216299

[28] Siddiquee, S. (2017). Recent advancements on the role of biologically active secondary metabolites from Aspergillus. In *New and Future Developments in Microbial Biotechnology and Bioengineering: Penicillium System Properties and Applications* (pp. 69–94). Elsevier. ISBN 9780444635013

[29] Mittra, B., Saha, A., Chowdhury, A. R., Pal, C., Mandal, S., Mukhopadhyay, S., Bandyopadhyay, S., & Majumder, H. K. (2000). Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. *Molecular Medicine, 6*, 527–541. https://doi.org/10.1007/bf03401792

[30] Espinosa, A., Paz-y-Miño-C, G., Santos, Y., Ma, H., Nadeau, M., Seeram, N. P., & Rowley, D. C. (2020). Anti-amebic effects of Chinese rhubarb (*Rheum palmatum*) leaves' extract, the anthraquinone rhein, and related compounds. *Heliyon, 6*. https://doi.org/10.1016/j.heliyon.2020.e03693

[31] Atanu, F. O., Rotimi, D., Ilesanmi, O. B., Al Malki, J. S., Batiha, G. E., & Idakwoji, P. A. (2022). Hydroethanolic extracts of Senna alata leaves possess antimalarial effects and reverse haematological and biochemical perturbation in Plasmodium berghei-infected mice. *Journal of Evidence-Based Integrative Medicine, 27*. https://doi.org/10.1177/2515690X221116407