

Article



Antiparasitic Potential of Methanol Extract of Brown Alga Sargassum polycystum (Phaeophyceae) and Its LC-MS/MS Metabolite Profiling

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Abstract: In Southeast Asian nations, cultured hybrid groupers are facing serious infestation by the marine parasitic leech Zeylanicobdella arugamensis (Annelida, Hirudinea). They attach to the hybrid groupers by sucking and biting on the surface of the skin, paving the way for secondary infection upon the fish. The objective of the study is to utilize the locally available seaweed to control the infestation of parasitic leeches. The methanol extracts of the brown alga Sargassum polycystum C. Agardh, 1824 (Phaeophyceae) from Sabah were prepared and investigated for antiparasitic efficacy against Z. arugamensis through in vitro bioassay. A total of 126 adult leeches from the host hybrid groupers were obtained from the fish hatchery. The parasitic leeches were treated with the methanol extracts of S. polycystum for 180 min by preparing five different dosages at concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL. The brown alga was found to have high antiparasitic efficacy, resulting in 100% leech mortality over a short period of time. It showed the highest antiparasitic efficacy (total mortality of leeches) in a short time limit of 0.96 ± 0.44 min, for 100 mg/mL of the extract. Observations on leech behavior in the positive control and the seaweed extract treatments showed vigorous swimming before mortality. LC-MS/MS analysis was used to reveal the phytochemical composition of the extract to understand the nature of the main components responsible for its antiparasitic activities. A total of 29 metabolites were identified via Q Exactive HF Orbitrap mass spectrometry, including two flavonoids (ephedrannin A and hinokiflavone), two organoarsenics (1-dimethylarsinoyl-heptadecane and cacodylic acid), four heterocyclic compounds, and two chlorophyll breakdown products. The presence of bioactive compounds could increase the mortality rate of parasitic leeches. Thus, this study concludes that the brown alga showed high efficacy in its antiparasitic activities and can be effectively applied for treatment in grouper aquaculture farms for sustainable aquaculture.

Keywords: aquaculture; parasites; antiparasitic activity; *Sargassum polycystum*; hybrid grouper; bioactive compounds; *Zeylanicobdella arugamensis*

1. Introduction

Drugs derived from aquatic organisms are known as "marine drugs." Since ancient times, these marine medications have been employed for various purposes [1–3]. Bergmann [1] published the first physiologically active marine natural product in late 1950. Subsequently, it was discovered in the late 1970s that marine plants and animals are genetically and biochemically distinct. Since then, over 15,000 natural compounds with different bioactivities have been identified from marine microorganisms, algae, and crustaceans [4]. Fatty acids, sterols, carotenoids, polysaccharides, dietary fibers, agar,

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/license s/by/4.0/). carrageenan, alginate, and phycocolloids are among the bioactive compounds that can be found in macroalgae. Scientists are focusing on a variety of bioactive chemicals found in seaweed because of their antiviral, antitumor, anti-inflammatory, antilipidemic, and other effects [5].

There are three main categories of macroalgae: brown algae (Phaeophyceae), green algae (Chlorophyta), and red algae (Rhodophyta). Brown algae comprise 1500–2000 species worldwide. The presence of a xanthophyll pigment known as fucoxanthin causes brown algae to have a yellowish or brown hue. *Sargassum* is one of the most common and most abundant brown algae, with more than 400 recognized species worldwide, and they are usually found in shallow waters of the subtropics and tropics [6]. One of the common species is *Sargassum polycystum* C. Agardh, 1824 (rough-stemmed sargassum), which belongs to the family Sargassaceae. It has filiform stalks, lanceolate leaves with serrated margins, and many vesicles [7]. *Sargassum polycystum* extract has gained popularity in the biomedical field because it includes fucoidan, which is known to have antioxidant and anticancer properties [8].

Malaysia's aquaculture industry makes a considerable contribution to the country's economy. The development of Malaysia's aquaculture sector is predicted to become one of the country's most important agricultural contributors, both as a source of foreign exchange and, more crucially, as a supply of animal protein. Cage-culturing fish is a way to boost productivity and it was introduced to Malaysia in the early 1970s [9]. In Malaysia, the major species of marine fish cultured in cages are groupers (*Epinephelus coioides, E. lanceolatus, E. fuscoguttatus*), snappers (*Lutjanus johnii, L. argentimaculatus, L. stellatus*), and seabass (*Lates calcarifer*) [10–12].

Grouper (Family: Serranidae, Subfamily: Epinephelinae) aquaculture is a significant portion of many countries' aquaculture production portfolios, particularly in Asia. In Malaysia, grouper aquaculture has been carried out in hatcheries and floating cages. There are two systems involved in grouper aquaculture. The first one is "system" culture, and the second one is "real" culture. In Sabah, *E. coioides* and *E. malabaricus* fry/fingerlings are raised through real culture [13]. The fry/fingerlings are usually imported from Thailand, the Philippines, or caught in local coastal waters [11]. Hybridization has been attempted by researchers in order to improve grouper quality and productivity. Hitherto, the most successful hybrid grouper combination is tiger grouper x giant grouper (TGGG), since it has a fast growth rate [14], has improved feeding efficiency, and has a higher survival rate [15].

Many fish producers in Asia have suffered significant financial losses as a result of disease outbreaks in cultured groupers [16,17]. The cultured groupers (*Epinephelus malabaricus*) experienced a higher number of infestations than wild ones [11]. Leeches, monogeneans, caligids, copepods, protozoans, didymozoid digeneans, nematodes, and isopods are some of the fish parasites that may plague the grouper culture sector [16-18].

Zeylanicobdella arugamensis de Silva, 1963 (Piscicolidae, Hirudinea), a parasitic marine leech, is one of the most harmful parasites found on the body surface of cultured hybrid groupers and other grouper species [18]. These leeches have striated bodies, a strong body wall, and two suckers which they utilize to feed and move. Due to heavy infestations of the leech *Z. arugamensis*, adult cultured orange-spotted grouper, *E. coioides*, have been reported to die three days after bleeding out. Thus, marine leeches are a threat to the aquaculture industry. Researchers have tested the effectiveness of freshwater and formalin bath treatments to eliminate marine leeches from infested fish [18, 19]. Furthermore, to avoid the re-infestation of leeches caused by the hatching of cocoons deposited on the walls of the fish tanks, aquaculture facilities have also attempted exposure of tanks to the blazing sun. However, all these measures seem less effective and life-threatening than other methods, especially formalin [19].

In the search for antiparasitic agents from natural products, researchers have recently turned their attention to marine sources, particularly seaweeds [20]. The goal of this study is to utilize *S. polycystum* extract to manage parasitic leech infestations and to use LC-MS profiling to identify the probable bioactive compounds responsible for antiparasitic actions.

2. Materials and Methods

2.1. Chemicals

Formalin (37% aqueous formaldehyde solution) was purchased from Sigma, Leica, Microsystem, and Germany. HPLC grade methanol and hexane were obtained from Merck (Darmstadt, Germany). Polyvinylidene fluoride (PVDF) syringe filters with 0.22 µm pore size and 13 mm diameter were purchased from Merck (Darmstadt, Germany).

2.2. Sample Collection

Fresh brown seaweed, *S. polycystum* (Figure 1), was collected in the morning manually during low tides on reef flats from the shore at the Outdoor Development Centre (ODEC), UMS (6.042911, 116.111421) in September 2021. The morphological identification was carried out and a voucher specimen (IPMB-A 01.00001) was deposited at the Marine Specimen Reference Collection Room of the Borneo Marine Research Institute, Kota Kinabalu. The collected sample was cleaned of unwanted debris including epiphytes, sand, pebbles, and shells by washing thoroughly with tap water.



Figure 1. Fresh Sargassum polycystum collected from Kota Kinabalu, Sabah, Malaysia.

2.3. Solvent Extraction

The blade, stipe, and vesicles of the seaweed were cleaned with distilled water before being dried in an oven for two days at 37 °C. The dried plant was ground into a fine powder using a mechanical grinder and stored in an airtight container. The extraction of dry seaweed powder was carried out using the maceration method [21] with slight modification. A total of 60 g and 600 mL of dry seaweed powder and methanol, respectively, were utilized in this single multistage extraction method. Sixty grams of dry seaweed powder was extracted using 300 mL of HPLC grade methanol over three days in an incubator benchtop shaker (25-30 °C, 120 rpm) (Orbicult IBS-NR-25-8, ESCO, Singapore). The mixture was vacuum-filtered through Whatman No. 1 filter paper. The residue that remained on the filter was collected in a conical flask. Then, the residue was added subsequently to another 300 mL of methanol, while the filtrate was stored in a Scott bottle. The residue was then extracted after three days. The mixture was vacuum-filtered to obtain the filtrate. A combination of filtrates from the first and the second filtration was concentrated using a vacuum rotary evaporator (R-215, BUCHI, Flawil, Switzerland). A freeze dryer was used to lyophilize the filtrate after it had been maintained at -80 °C for 24 h (Freezon 12, Labconco, Kansas City, MO, USA). After the filtrate was lyophilized, the extract (5.10 g) was kept for future research in the freezer.

2.4. Antiparasitic Bioassay

Parasitic leeches infesting hybrid grouper (Figure 2) were collected from the aquaculture facilities. Based on their morphological characteristics, the leeches were identified [10].



Figure 2. Hybrid grouper infested by leeches (indicated by red arrows) all over the body, abundantly attached on the fin.

Adult leeches were selected and divided into seven groups and each group consisted of six leeches in a Petri dish. One milliliter of extract solution for each group was used and placed in a Petri dish. Then, the leeches were exposed to the normal control, positive control, and different concentrations of seaweed extract solution.

Group 1: Normal control, treated with seawater groups only.

Group 2: Positive control, treated with formalin (0.25% v/v) solution.

Groups 3, 4, 5, 6, and 7: Treated with 6.25, 12.5, 25, 50, and 100 mg/mL of the methanol extracts of *S. polycystum*, respectively (Figure 3). The solution was produced using the serial dilution technique.



Figure 3. Experimental *Zeylanicobdella arugamensis*, (**A**) normal control, (**B**) positive control, (**C–H**) *Sargassum polycystum* methanol extract-administered group.

During the challenge, mortality time was recorded using a stopwatch for 720 min [21]. The experiment was performed in triplicate.

2.5. Observation of Leech Behavior

The changes in the behavior of leeches were observed visually after exposure to formalin and different concentrations of seaweed extract, and the results are compared to the normal control group.

2.6. Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) Acquisition

LC-MS/MS analysis was performed using the Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) coupled with the Thermo Scientific Q Exactive HF Orbitrap mass spectrometry system (Thermo Fisher Scientific, Waltham, MA, USA) as described in [22,23]. A Thermo Syncronis C18 column (2.1 mm × 100 mm × 1.7 μ m; Thermo Fisher Scientific, Waltham, MA, USA) was used for liquid chromatography and maintained at 55 °C at a flow rate of 450 μ L/min during analysis. All instrumental settings and elution gradients were described earlier in [22], and instrument calibration was performed before the analysis.

2.7. Data Analysis

The acquired data were processed and analyzed using MZmine 3.1.0 software [24]. Briefly, raw mass spectrometry data were processed for mass detection, chromatogram building, smoothing, and alignment, before putative identification. Identification of metabolites was carried out with MZmine 3 and the processed data were matched against the Human Metabolome Database (HMDB) [25,26], LIPID MAPS[®] Structure Database (LMSD) [27], Global Natural Products Social Molecular Networking (GNPS) library [28], and ChemSpider and matching tolerance was limited to 5 ppm mass error.

2.8. Statistical Analysis

Data analysis was carried out using the IBM SPSS Statistics 25 Window package (IBM, Armonk, NY, US). Significant differences between groups were investigated using a one-way analysis of variance (ANOVA) followed by Tukey's test. All data points were shown as mean \pm standard deviation (S.D.). *p* values = 0.05 were viewed as significant [21].

3. Results

3.1. Antiparasitic Properties of Sargassum polycystum

Table 1 shows the *Z. arugamensis* mortality time when treated with formalin and extracts. The groups that were treated with seaweed extract showed the antiparasitic effect in a dose-dependent manner. In comparison to the doses of 50, 25, 12.5, and 6.25 mg/mL of the methanol extract, the time required for the parasitic leeches to die was shorter at 100 mg/mL (Figure 3). No mortality was noticed in the negative control group throughout the 180 min observation.

Table 1. Mortality time of the leeches at different concentrations of methanol extract of *S. polycystum*. Each value represents the mean ± S.D of 6 parasitic leeches per group.

N	Crown	Mortality Time (min)		
INO.	Group	Mean ± S.D.		
1	*Normal control	0.00 ± 00		
2	[#] Positive control	1.59 ± 0.30 °		
3	6.25 (mg/mL)	30.18 ± 7.69 ^{a,b}		
4	12.5 (mg/mL)	11.10 ± 2.52 ^{a,b,c}		
5	25 (mg/mL)	6.93 ± 2.44 ^{a,b,c,d}		
6	50 (mg/mL)	2.77 ± 1.40 ^{a,b,c,d,e}		
7	100 (mg/mL)	0.93 ± 0.44 a,b,c,d,e,f,g		

^a Significance at p = 0.05 compared with the normal control; ^b significance at p = 0.05 compared with the positive control; ^c significance at p = 0.05 compared with 6.25 mg/mL seaweed extract; ^d signifi-

cance at p = 0.05 compared with 12.5 mg/mL seaweed extract; ^e significance at p = 0.05 compared with 25 mg/mL seaweed extract; ^f significance at p = 0.05 compared with 50 mg/mL seaweed extract; ^g significance at p = 0.05 compared with 100 mg/mL seaweed extract; * seawater; * 0.25% (v/v) formalin.

3.2. Behavior of Z. arugamensis

The leeches in the seawater moved in an orderly manner by using their anterior and posterior suckers. The leeches were firmly attached to the Petri dish using their suckers. Leeches that had been exposed to formalin initially swam vigorously before deteriorating slowly and eventually ceasing to move. Some of the parasitic leeches were able to attach with the anterior or posterior sucker at the bottom of the Petri dish before they died, compared to the extract-treated group. Leeches exposed to methanol extract of *S. polycystum* at various doses exhibited disorganized movement and were unable to use their anterior or posterior suckers to move. The leeches exhibited vigorous swimming behavior in a zig-zag pattern, then, the swimming gradually halted as the leeches were paralyzed and died. No movements were observed after physical touch.

3.3. Physicochemical Parameters of Leeches Treated with Solutions

The water quality parameters of the control group and seaweed extract group solutions applied for the antiparasitic assays are shown in Table 2. The pH value of the extract group decreased as the concentration of extract increased. The presence of bioactive compounds with acidic nature in the extract group resulted in slightly acidic conditions. The dissolved oxygen for all groups was almost the same and ranges from 7.85 to 8.08 mg/mL. The highest concentration of the extract group shows high salinity compared to other groups.

Group	pН	Salinity (ppt)	Dissolved Oxygen (mg/mL)	Temperature (°C)
* Normal control	6.53	23.1	7.91	26.2
[#] Positive control	6.36	15.3	7.90	26.2
6.25 (mg/mL)	6.27	24.0	8.08	25.5
12.5 (mg/mL)	6.23	18.5	7.81	25.1
25 (mg/mL)	6.01	29.1	8.04	25.2
50 (mg/mL)	5.76	34.7	8.03	25.4
100 (mg/mL)	5.43	46.9	7.85	25.9

Table 2. Water quality parameters of the solutions applied for the fish treatment.

* Seawater; # 0.25% (v/v) formalin.

3.4. LC-MS Analysis and Metabolite Identification

In the present study, a total of 29 metabolites were identified via Q Exactive HF Orbitrap mass spectrometry (Table 3). Among these 29 metabolites, there are two flavonoids (ephedrannin A and hinokiflavone), two organoarsenics (1-dimethylarsinoyl-heptadecane and cacodylic acid), three heterocyclic compounds, and two chlorophyll breakdown products (Figure 4). In addition, a steroidal compound, salvianolic acid (No. 28), was detected as well. However, identification of underivatized steroidal compounds via tandem mass spectrometry is impossible and any putative identity could be misleading due to their stable 4-ring skeleton and diverse stereoisomerisms [29]. Thus, the putative identity of the steroidal compound was masked, and only provided with the detected m/z and formula.

No.	Matched Metabolites	Molecular Formula	m/z	Mass Error (ppm)	Class
1	2-Aminoheptanoic acid	C7H15NO2	146.1176	0.42	Amino Acid
2	5-Aminopentanoic acid	C5H11NO2	118.0866	3.08	Amino Acid
3	1-Hydroxy-5-phenyl-3-pentanone	$C_{11}H_{14}O_2$	179.1069	1.47	Aromatic
4	2-(4-Bromophenyl) butanoic acid	$C_{10}H_{11}BrO_2$	243.0005	-3.88	Aromatic
5	2-Anilino-3-chloro-1,4-naphthoquinone	$C_{16}H_{10}ClNO_2$	284.0466	-2.26	Aromatic
6	2-Benzoyl-5-chlorobenzoic acid	C14H9ClO3	261.0306	-2.46	Aromatic
7	3-(2,6-Diisopropylphenyl)-1-{1-[3-(2,5-dimethylp henyl)-4-oxo-3,4-dihydro-2-quinazolinyl]ethyl}- 1-(2-phenylethyl)urea	C39H44N4O2	601.3529	-1.23	Aromatic
8	6-(3-Ethoxy-4-hydroxyphenyl)-8-[(4-methylphen yl)amino]-1,3,7,9-tetraoxo-6a-phenyl-3,3a,4,6,6a, 7,8,9,9a,10,10a,10b-dodecahydroisoindolo [5,6-e]isoindole-2(1H)-carboxamide	C36H34N4O7	635.2504	0.73	Aromatic
9	Diphenyl sulfoxide	C12H10OS	203.0529	2.28	Aromatic
10	Harderoporphyrin	C35H36N4O6	609.2706	-0.23	Chlorophyll Breakdown Product
11	Pyrophaeophorbide a	C33H34N4O3	535.2708	0.86	Chlorophyll Breakdown Product
12	11-Amino-undecanoic acid	C11H23NO2	202.1803	0.80	Fatty Acyl
13	11-Oxo-undeca-5,8-dienoic acid	$C_{11}H_{16}O_3$	197.1175	1.84	Fatty Acyl
14	Erucamide	C22H43NO	338.3416	-0.41	Fatty Acyl
15	3-(7-tetradecenoyloxy)-dodecanoic acid	$C_{26}H_{48}O_4$	425.3625	-0.09	Fatty Acyl
16	Termitomycesphin F	C43H83NO10	774.6094	0.60	Fatty Acyl
17	Ephedrannin A	C30H20O11	557.1078	-0.07	Flavonoid
18	Hinokiflavone	C30H18O10	539.0977	0.86	Flavonoid
19	Ectoine	$C_{6}H_{10}N_{2}O_{2}$	143.0815	0.43	Heterocyclic
20	Erinapyrone C	$C_8H_{10}O_5$	187.0603	1.40	Heterocyclic
21	Stachydrine	C7H13NO2	144.1020	1.13	Heterocyclic
22	1-Dimethylarsinoyl-heptadecane	C19H41OAs	361.2446	0.17	Organoarsenic
23	Cacodylic acid	C2H7AsO2	138.9736	1.17	Organoarsenic
24	5-Bromo-2-hydroxy-N,3-dimethylbenzamide	C9H10BrNO2	243.9971	1.49	Phenolic
25	Ethephon	C ₂ H ₆ ClO ₃ P	144.9822	4.59	Plant Growth Regulator
26	N-Benzyl-N~2~-isobutyl-N-{[1-(3-methoxybenzy l)-1H-pyrrol-2-yl]methyl}-N~2~-(2-naphthylsulf onyl)glycinamide	C36H39N3O4S	610.2743	1.58	Polyaromatic
27	Salvianolic acid L	C36H30O16	719.1609	0.36	Polyphenolic
28	Steroidal compound	C29H49NO2	444.3839	0.81	Steroid
29	α -Carboxydimethyloctylhydroxychroman	C24H38O4	391.2842	-0.10	Vitamin E

Table 3. Matched metabolites in the methanol extract of <i>Sargassum polycystum</i> .



Figure 4. Chemical structures of the 28 secondary metabolites detected in the *Sargassum polycystum* extract (see Table 3 for names of the metabolites).

4. Discussion

The seaweed *S. polycystum* is known for its antibacterial activity [30] and antioxidant and anticancer properties [8]. One of the brown seaweed compounds, fucoidan (fu-

cose-containing sulfated polysaccharide), has been extensively studied since it exhibits many biological and pharmacological properties such as anticoagulant/antithrombotic, antitumor, antivirus, and anti-inflammatory effects [31].

In this study on treatment, seaweed extract killed parasitic leeches effectively at the highest concentration (100 mg/mL) in vitro within a shorter time (0.96 \pm 0.44 min). A previous study on neem plant (*Azadirachta indica*) leaf extract showed a strong antiparasitic effect on *Z. arugamensis* when administered in vitro at a concentration of 100 mg/mL within 6.45 \pm 0.45 min [22] compared to 100 mg/mL of *Dillenia suffruticosa* extract at which the leech mortality time was 14.39 \pm 3.75 min [32]. Likewise, the plant extract was tested against the leech *Limnatis nilotica*. The treatment groups of common grape vine *Vitis vinifera* methanol extract (300 and 600 mg) on *L. nilotica* reported the death times of 260 \pm 63 and 200 \pm 50 min, respectively [33]. With another plant, *Nephrolepis biserrata*, *Z. arugamensis* was paralyzed after administering 25, 50, and 100 mg/mL of the extract with an average mortality time of 25.11 \pm 3.26, 11.91 \pm 0.99, and 4.88 \pm 0.50 min, respectively [21]. These corresponding results indicate that the antiparasitic efficacies of natural product extract against leeches are vital for sustainable aquaculture.

In the present study, a strong antiparasitic effect of formalin at 0.25 mg/mL was observed *in vitro*. Previous studies have demonstrated the antiparasitic effectiveness of formalin on adult leeches with particularly high success in vitro [18]. Formalin has also been used to treat another type of fish parasite. For example, Thing et al. [34] demonstrated that exposure to 100 and 200 ppm formalin killed pathogenic isopods, *Caecognathia coralliophila*, in vitro within 24 h. In the antiparasitic test, 250 and 400 ppm of formalin were also found to be effective to remove *Benedenia seriolae* and *Zeuxapta seriolae* monogeneans from the yellowtail kingfish, *Seriola lalandi* [35]. In addition, the antiparasitic effectiveness of formalin in the freshwater environment to control the protozoan parasite *Ichthyophthirius multifiliis* had also been reported [36]. Maria Andrade-Porto et al. [37] revealed that formalin was effective against the monogenean *Dawestrema cycloancistrium* at high concentrations (440 and 550 mg/L) and short exposure time (1 h). So, it is assumed that formalin is an effective chemical to control parasites but has adverse effects of its use.

The LC-MS/MS analysis of a methanol extract of *S. polycystum* indicated the presence bioactive flavonoids (ephedrannin A and hinokiflavone), organoarsernics of (1-dimethylarsinoyl-heptadecane and cacodylic acid), and other potential bioactive metabolites which possessed heterocyclic, aromatic, or phenolic functional groups. Ephedrannin A, also known as a dimeric proanthocyanidin, was reported to possess anti-inflammation properties [38]. On the other hand, hinokiflavone is known for cancer inhibition, anti-inflammation, and antiparasitic properties [39–41]. In an antiparasitic study [39], hinokiflavone significantly inhibited the growth of Leishmania donovani and *Plasmodium falciparum* with IC50 of 2.9 and 2.3 μ M, respectively. The presence of lipid-soluble organoarsenic metabolites in marine fishes and algae is common, but yet to be reported in any terrestrial organisms [42-44]. Arsenic-containing hydrocarbon, such as 1-dimethylarsinoyl-heptadecane and cacodylic acid, is reported to possess neurotoxicity [44]. Furthermore, cacodylic acid, also known as Agent Blue, is a commonly used active compound in herbicides [45,46]. Overall, the metabolites found in the methanolic extracts of S. polycystum are correlated with the antiparasitic effect. However, further study on the quantitative evaluation of the detected bioactive compounds is essential.

Most studies published to date have concentrated solely on the effectiveness of medicinal plants for the removal of parasitic marine leeches from host fish. Seaweed extract could be a better alternative in controlling leech infestation as it has unique biological features and is a natural component of the marine environment. Therefore, in the present study, the effect of the seaweed extract on parasitic leeches was investigated. Previously, aqueous seaweed extract of the common tropical seaweeds was shown to be ineffective in killing adults of the monogenean ectoparasite *Neobenedenia* sp. [20]. However, there are materials describing sulfated polysaccharides (SPSs), found in marine hydrobionts including algae and invertebrates, as prospective treatments and preventative measures for protozoa and helminthiasis [47–49].

5. Conclusions

This study has revealed the effects of a natural treatment utilizing the seaweed *S. polycystum* to kill adult leeches. It took just 0.96 ± 0.44 min per 100 mg/mL concentration of the seaweed extract to kill *Z. arugamensis*. The phytochemical components of *S. polycystum* methanolic extract contained 29 metabolites, including two flavonoids (ephedrannin A and hinokiflavone), two organoarsenics (1-dimethylarsinoyl-heptadecane and cacodylic acid), three heterocyclic compounds, and two chlorophyll breakdown products. Notably, there are no treatments against leeches from marine sources and currently available treatments only focus on terrestrial plant extracts. The use of marine sources could be a better alternative for the treatment of infested cultured marine fish. Seaweed extracts can be applied directly in closed systems, however, treatment waste disposal should be considered to avoid water pollution. More studies are needed for the quantitative evaluation of the detected bioactive compounds and to explore the mechanisms of bioactive compounds from the extract as well.

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