diversity

MDPI

Article

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

Fatin Khairah Haron [1], Muhammad Dawood Shah [1], Yoong Soon Yong [2], Jen Kit Tan [3], Mohammad Tamrin Mohamad Lal [1] and Balu Alagar Venmathi Maran [1,*]

[1] Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu 88450, Malaysia
[2] Laboratory Center, Xiamen University Malaysia, Jalan Sunsuria, Bandar Sunsuria, Sepang 43900, Malaysia
[3] Department of Biochemistry, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, Kuala Lumpur 56000, Malaysia
* Correspondence: bavmaran@ums.edu.my; Tel.: +60-16-2096905

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*

Citation: Haron, F.K.; Shah, M.D.; Yong, Y.S.; Tan, J.K.; Lal, M.T.M.; Venmathi Maran, B.A. Antiparasitic Potential of Methanol Extract of Brown Alga *Sargassum polycystum* (Phaeophyceae) and Its LC-MS/MS Metabolite Profiling. *Diversity* **2022**, *14*, 796. https://doi.org/10.3390/d14100796

Academic Editor: Jesús Fernando Ayala-Zavala

Received: 20 August 2022
Accepted: 21 September 2022
Published: 24 September 2022

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, 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.

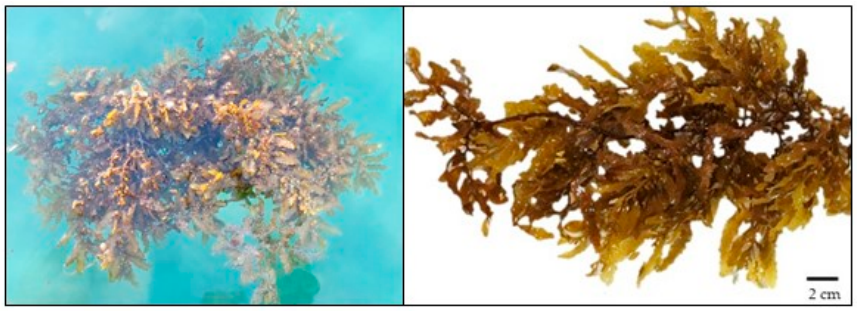

Figure 1. Fresh *Sargassum polycystum* collected from Kota Kinabalu, Sabah, Malaysia.

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].

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 Figure 3A.

Group 2: Positive control, treated with formalin (0.25% *v/v*) solution Figure 3B.

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 3C–H). The solution was produced using the serial dilution technique.

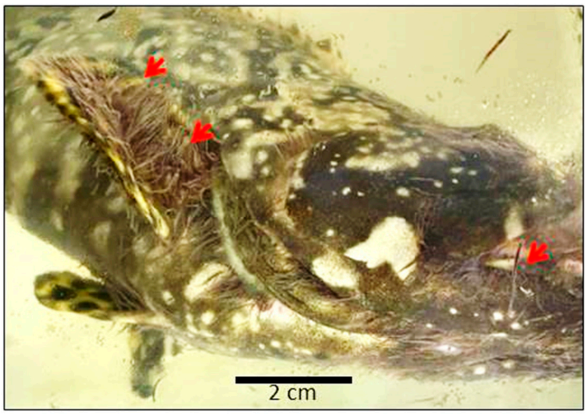

Figure 2. Hybrid grouper infested by leeches (indicated by red arrows) all over the body, abundantly attached on the fin.

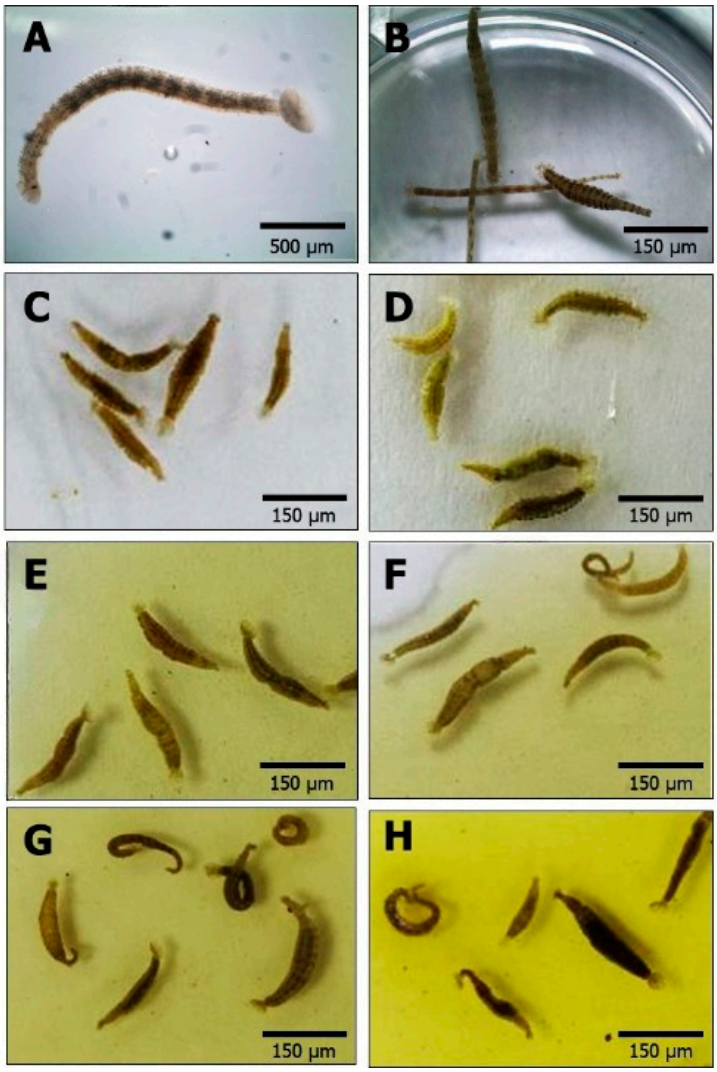

Figure 3. Experimental *Zeylanicobdella arugamensis*, (**A**) normal control, (**B**) positive control, (**C**–**H**) *Sargassum polycystum* methanol extract-administered group.

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 µm; Thermo Fisher Scientific, Waltham, MA, USA) was used for liquid chromatography and maintained at 55 °C at a flow rate of 450 µL/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, USA). 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 ± 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.

No.	Group	Mortality Time (min) Mean ± S.D.
1	* Normal control	0.00 ± 00
2	# Positive control	1.59 ± 0.30 [a]
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] significance 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.

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

Group	pH	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

* 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.

Table 3. Matched metabolites in the methanol extract of *Sargassum polycystum*.

No.	Matched Metabolites	Molecular Formula	*m/z*	Mass Error (ppm)	Class
1	2-Aminoheptanoic acid	$C_7H_{15}NO_2$	146.1176	0.42	Amino Acid
2	5-Aminopentanoic acid	$C_5H_{11}NO_2$	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	$C_{14}H_9ClO_3$	261.0306	−2.46	Aromatic
7	3-(2,6-Diisopropylphenyl)-1-{1-[3-(2,5-dimethylphenyl)-4-oxo-3,4-dihydro-2-quinazolinyl]ethyl}-1-(2-phenylethyl)urea	$C_{39}H_{44}N_4O_2$	601.3529	−1.23	Aromatic
8	6-(3-Ethoxy-4-hydroxyphenyl)-8-[(4-methylphenyl)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	$C_{36}H_{34}N_4O_7$	635.2504	0.73	Aromatic
9	Diphenyl sulfoxide	$C_{12}H_{10}OS$	203.0529	2.28	Aromatic
10	Harderoporphyrin	$C_{35}H_{36}N_4O_6$	609.2706	−0.23	Chlorophyll Breakdown Product
11	Pyrophaeophorbide a	$C_{33}H_{34}N_4O_3$	535.2708	0.86	Chlorophyll Breakdown Product
12	11-Amino-undecanoic acid	$C_{11}H_{23}NO_2$	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	$C_{22}H_{43}NO$	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	$C_{43}H_{83}NO_{10}$	774.6094	0.60	Fatty Acyl
17	Ephedrannin A	$C_{30}H_{20}O_{11}$	557.1078	−0.07	Flavonoid
18	Hinokiflavone	$C_{30}H_{18}O_{10}$	539.0977	0.86	Flavonoid
19	Ectoine	$C_6H_{10}N_2O_2$	143.0815	0.43	Heterocyclic
20	Erinapyrone C	$C_8H_{10}O_5$	187.0603	1.40	Heterocyclic
21	Stachydrine	$C_7H_{13}NO_2$	144.1020	1.13	Heterocyclic
22	1-Dimethylarsinoyl-heptadecane	$C_{19}H_{41}OAs$	361.2446	0.17	Organoarsenic
23	Cacodylic acid	$C_2H_7AsO_2$	138.9736	1.17	Organoarsenic
24	5-Bromo-2-hydroxy-N,3-dimethylbenzamide	$C_9H_{10}BrNO_2$	243.9971	1.49	Phenolic
25	Ethephon	$C_2H_6ClO_3P$	144.9822	4.59	Plant Growth Regulator
26	N-Benzyl-N~2~-isobutyl-N-{[1-(3-methoxybenzyl)-1H-pyrrol-2-yl]methyl}-N~2~-(2-naphthylsulfonyl)glycinamide	$C_{36}H_{39}N_3O_4S$	610.2743	1.58	Polyaromatic
27	Salvianolic acid L	$C_{36}H_{30}O_{16}$	719.1609	0.36	Polyphenolic
28	Steroidal compound	$C_{29}H_{49}NO_2$	444.3839	0.81	Steroid
29	α-Carboxydimethyloctylhydroxychroman	$C_{24}H_{38}O_4$	391.2842	−0.10	Vitamin E

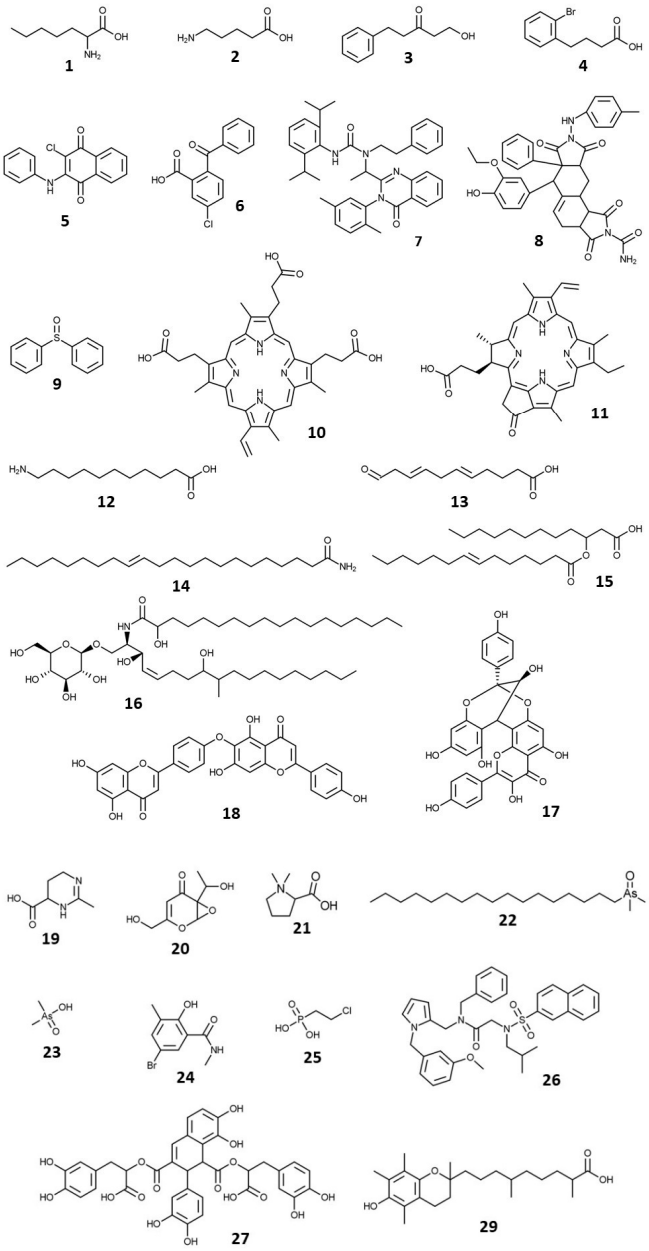

Figure 4. Chemical structures of the 28 secondary metabolites detected in the *Sargassum polycystum* extract (see Table 3 for names of the metabolites).

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 (fucose-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 ± 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 ± 0.45 min [22] compared to 100 mg/mL of *Dillenia suffruticosa* extract at which the leech mortality time was 14.39 ± 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 ± 63 and 200 ± 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 ± 3.26, 11.91 ± 0.99, and 4.88 ± 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]. Few studies. [37,38] 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 of bioactive flavonoids (ephedrannin A and hinokiflavone), organoarsernics (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 [39]. On the other hand, hinokiflavone is known for cancer inhibition, anti-inflammation, and antiparasitic properties [39–41]. In an antiparasitic study [40], hinokiflavone significantly inhibited the growth of *Leishmania donovani* and *Plasmodium falciparum* with IC50 of 2.9 and 2.3 μM, 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.

Author Contributions: Conceptualization, supervision, funding acquisition, correspondence, B.A.V.M.; methodology, investigation, B.A.V.M., F.K.H. and M.D.S.; formal analysis and data curation, B.A.V.M., M.D.S., Y.S.Y. and J.K.T.; writing—original draft preparation, F.K.H. and B.A.V.M.; writing—review and editing, B.A.V.M., M.D.S., M.T.M.L., Y.S.Y. and J.K.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FRGS/1/2018/STG03/UMS/02/2 and UMS project grant number SDN0073-2019.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank the UMS-BMRI hatchery manager and staff for their help in the sample collection.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bergmann, W. Contributions to the study of marine products: II. The sterols of mollusks. *J. Biol. Chem.* **1934**, *104*, 317–328. [CrossRef]
2. Shah, M.D.; Venmathi Maran, B.A.; Shaleh, S.R.M.; Zuldin, W.H.; Gnanaraj, C.; Yong, Y.S. Therapeutic potential and nutraceutical profiling of North Bornean seaweeds: A review. *Mar. Drugs* **2022**, *20*, 101. [CrossRef] [PubMed]
3. Venmathi Maran, B.A.; Iqbal, M.; Gangadaran, P.; Ahn, B.C.; Rao, P.V.; Shah, M.D. Hepatoprotective potential of Malaysian medicinal plants: A review on phytochemicals, oxidative stress, and antioxidant mechanisms. *Molecules* **2022**, *27*, 1533. [CrossRef] [PubMed]
4. Salomon, C.E.; Magarvey, N.A.; Sherman, D.H. Merging the potential of microbial genetics with biological and chemical diversity: An even brighter future for marine natural product drug discovery. *Nat. Prod. Rep.* **2004**, *21*, 105–121. [CrossRef] [PubMed]
5. Pal, A.; Kamthania, M.C.; Kumar, A. Bioactive compounds and properties of seaweeds-A review. *Open Access Libr. J.* **2014**, *1*, 1–17. [CrossRef]
6. Chiang, Y.-M.; Yoshida, T.; Ajisaka, T.; Trono, G., Jr.; Tseng, C.K.; Baoren, L. Distribution and variation in *Sargassum polycystum* C. A. Agardh (Fucales, Phaeophyta). In *Taxonomy of Economic Seaweeds with Reference to Some Pacific and Western Atlantic Species*; California Sea Grant College: San Diego, CA, USA, 1992; pp. 35–42.
7. Ajisaka, T.; Phang, S.M.; Yoshida, T. Preliminary report of *Sargassum* species collected from Malaysian coasts. In *Taxonomy of Economic Seaweeds with Reference to Some Pacific Species*; Abbott, I.A., Ed.; California Sea Grant College System: San Diego, CA, USA, 1999; pp. 23–41.
8. Palanisamy, S.; Vinosha, M.; Marudhupandi, T.; Rajasekar, P.; Prabhu, N.M. Isolation of fucoidan from *Sargassum polycystum* brown algae: Structural characterization, in vitro antioxidant and anticancer activity. *Int. J. Biol. Macromol.* **2017**, *102*, 405–412. [CrossRef]
9. Chua, T.E.; Teng, S.K. Floating fishpens for rearing fishes in coastal waters, reservoirs and mining pools in Malaysia. In *Fisheries Bulletin No.2*; Ministry of Agriculture: Kuala Lumpur, Malaysia, 1977; Volume 20, pp. 1–36.
10. Leong, T.-S.; Wong, S.-Y. A comparative study of the parasite fauna of wild and cultured grouper (*Epinephelus malabaricus* Bloch et Schneider) in Malaysia. *Aquaculture* **1988**, *68*, 203–207. [CrossRef]
11. Liong, P.C.; Hanafi, H.B.; Merican, Z.O.; Nagaraj, G. Aquaculture development in Malaysia. In Proceedings of the Seminar on Aquaculture Development in Southeast Asia, Iloilo City, Philippines, 8–12 September 1987; Juario, J.V., Benitez, L.V., Eds.; SEAFDEC, Aquaculture Department: Iloilo City, Philippines, 1987; pp. 73–90.
12. Kua, B.C.; Azmi, M.A.; Hamid, N.K.A. Life cycle of the marine leech (*Zeylanicobdella arugamensis*) isolated from sea bass (*Lates calcarifer*) under laboratory conditions. *Aquaculture* **2010**, *302*, 153–157. [CrossRef]
13. Sadovy, Y. Regional Survey for Fry/Fingerling Supply and Current Practices for Grouper Mariculture: Evaluating Current Status and Long-Term Prospects for Grouper Mariculture in Southeast Asia. In *Final Report to the Collaborative APEC Grouper Research and Development Network (FWG 01/99)*; Hong Kong University Press: Hong Kong, China, 2000.
14. Ch'Ng, C.L.; Senoo, S. Egg and larval development of a new hybrid grouper, Tiger grouper *Epinephelus fuscoguttatus* x Giant grouper *E. lanceolatus*. *Aquac. Sci.* **2008**, *56*, 505–512.

15. Othman, A.R.; Kawamura, G.; Senoo, S.; Ching, F.F. Effects of different salinities on growth, feeding performance and plasma cortisol level in hybrid TGGG (Tiger grouper, *Epinephelus fuscoguttatus* x Giant grouper, *Epinephelus lanceolatus*) juveniles. *Int. Res. J. Biol. Sci.* **2015**, *4*, 15–20.
16. Venmathi Maran, B.A.; Leong, T.S.; Ohtsuka, S.; Nagasawa, K. Records of *Caligus* (Crustacea: Copepoda: Caligidae) from marine fish cultured in floating cages in Malaysia with a redescription of the male of *Caligus longipedis* Bassett-Smith, 1898. *Zool. Stud.* **2009**, *48*, 797–807.
17. Gopalakrishnan, A.; Venmathi Maran, B.A.; Puvanendran, V.; Rajkumar, M.; Balasubramanian, T.; Ferguson, H. Neoplasia in the Indian oil sardine, *Sardinella longiceps* (Valenciennes), and the Great barracuda, *Sphyraena barracuda* (Edwards), from the south-east coast of India. *J. Fish Dis.* **2011**, *34*, 881–885. [CrossRef] [PubMed]
18. Cruz-Lacierda, E.R.; Toledo, J.D.; Tan-Fermin, J.D.; Burreson, E.M. Marine leech (*Zeylanicobdella arugamensis*) infestation in cultured Orange-spotted grouper, *Epinephelus coioides*. *Aquaculture* **2000**, *185*, 191–196. [CrossRef]
19. Leal, J.F.; Neves, M.G.P.M.S.; Santos, E.B.H.; Esteves, V.I. Use of formalin in intensive aquaculture: Properties, application and effects on fish and water quality. *Rev. Aquac.* **2016**, *10*, 281–295. [CrossRef]
20. Hutson, K.S.; Mata, L.; Paul, N.A.; De Nys, R. Seaweed extracts as a natural control against the monogenean ectoparasite, *Neobenedenia* sp., infecting farmed barramundi (*Lates calcarifer*). *Int. J. Parasitol.* **2012**, *42*, 1135–1141. [CrossRef]
21. Ayoub, Z.; Malik, S.B.; Mehta, A. In vitro and in vivo anti-cancer efficacy of *Adiantum capillus-veneris* L. against some selected human cancer cell lines and on EAC mouse model. *Int. J. Pharm. Sci. Drug Res.* **2020**, *12*, 267–274. [CrossRef]
22. 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. Antiparasitic potential of *Nephrolepis biserrata* methanol extract against the parasitic leech *Zeylanicobdella arugamensis* (Hirudinea) and LC-QTOF analysis. *Sci. Rep.* **2020**, *10*, 22091. [CrossRef]
23. Venmathi Maran, B.A.; Josmeh, D.; Tan, J.K.; Yong, Y.S.; Shah, M.D. Efficacy of the aqueous extract of *Azadirachta indica* against the marine parasitic leech and its phytochemical profiling. *Molecules* **2021**, *26*, 1908. [CrossRef]
24. Shah, M.D.; Venmathi Maran, B.A.; Tan, J.K.; Yong, Y.S.; Ching, F.F.; Shaleh, S.R.M.; Shapawi, R. The anti-leech potential of the solvent extract of Bornean neem leaves and ultra-high performance liquid chromatography-high-resolution mass spectrometry profiling. *J. King Saud Univ. Sci.* **2021**, *33*, 101541. [CrossRef]
25. Pluskal, T.; Castillo, S.; Villar-Briones, A.; Orešič, M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinform.* **2010**, *11*, 395. [CrossRef]
26. Wishart, D.S.; Feunang, Y.D.; Marcu, A.; Guo, A.C.; Liang, K.; Vázquez-Fresno, R.; Sajed, T.; Johnson, D.; Li, C.; Karu, N.; et al. HMDB 4.0: The human metabolome database for 2018. *Nucleic Acids Res.* **2018**, *46*, D608–D617. [CrossRef] [PubMed]
27. Wishart, D.S.; Guo, A.; Oler, E.; Wang, F.; Anjum, A.; Peters, H.; Dizon, R.; Sayeeda, Z.; Tian, S.; Lee, B.L.; et al. HMDB 5.0: The human metabolome database for 2022. *Nucleic Acids Res.* **2022**, *50*, D622–D631. [CrossRef] [PubMed]
28. Sud, M.; Fahy, E.; Cotter, D.; Brown, A.; Dennis, E.A.; Glass, C.K.; Merrill, A.H.; Murphy, R.C.; Raetz, C.R.H.; Russell, D.W.; et al. LMSD: LIPID MAPS structure database. *Nucleic Acids Res.* **2007**, *35*, D527–D532. [CrossRef]
29. Wang, M.; Carver, J.J.; Phelan, V.V.; Sanchez, L.M.; Garg, N.; Peng, Y.; Nguyen, D.D.; Watrous, J.; Kapono, C.A.; Luzzatto-Knaan, T.; et al. Sharing and community curation of mass spectrometry data with global natural products social molecular networking. *Nat. Biotechnol.* **2016**, *34*, 828–837. [CrossRef] [PubMed]
30. Murphy, R.C. *Tandem Mass Spectrometry of Lipids: Molecular Analysis of Complex Lipids*; Gaskell, S.J., Ed.; The Royal Society of Chemistry: Piccadilly, UK, 2015; ISBN 9781849738279.
31. Chiao-Wei, C.; Ling, H.S.; Wong, C.-L. Antibacterial activity of *Sargassum polycystum* C. Agardh and *Padina australis* Hauck (Phaeophyceae). *Afr. J. Biotechnol.* **2011**, *10*, 14125–14131. [CrossRef]
32. Li, B.; Lu, F.; Wei, X.; Zhao, R. Fucoidan: Structure and bioactivity. *Molecules* **2008**, *13*, 1671–1695. [CrossRef] [PubMed]
33. Shah, M.D.; Venmathi Maran, B.A.; Iqbal, M.; Ching, F.F.; Lal, M.M.T.; Othman, R.; Shapawi, R. Antiparasitic activity of the medicinal plant *Dillenia suffruticosa* against the marine leech *Zeylanicobdella arugamensis* (Hirudinea) and its phytochemical composition. *Aquac. Res.* **2020**, *51*, 215–221. [CrossRef]
34. Gholami-Ahangaran, M.; Bahmani, M.; Zia-Jahromi, N. Comparing of anti-leech effect of *Vitis vinifera* L. plant and niclosamide on different forms of *Limnatis nilotica*. *World Appl. Sci. J.* **2012**, *20*, 1082–1085. [CrossRef]
35. Thing, C.Y.; Ransangan, J.; Hatai, K. Antiparasitic effect of formalin, trichlorfon, hydrogen peroxide, and copper sulfate on the parasitic isopod *Caecognathia coralliophila*. *Fish Pathol.* **2016**, *51*, 125–127. [CrossRef]
36. Sharp, N.J.; Diggles, B.K.; Poortenaar, C.W.; Willis, T.J. Efficacy of aqui-s, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi lalandi* in New Zealand. *Aquaculture* **2004**, *236*, 67–83. [CrossRef]
37. Rowland, S.J.; Mifsud, C.; Nixon, M.; Read, P.; Landos, M. Use of formalin and copper to control ichthyophthiriosis in the Australian freshwater fish silver perch (*Bidyanus bidyanus* Mitchell). *Aquac. Res.* **2008**, *40*, 44–54. [CrossRef]
38. Andrade-Porto, S.M.; Affonso, E.G.; Kochhann, D.; Malta, J.C.O.; Roque, R.; Ono, E.A.; Araújo, C.S.O.; Tavares-Dias, M. Antiparasitic efficacy and blood effects of formalin on *Arapaima gigas* (Pisces: Arapaimidae). *Aquaculture* **2017**, *479*, 38–44. [CrossRef]
39. Kim, I.S.; Park, Y.J.; Yoon, S.J.; Lee, H.B. Ephedrannin A and B from roots of *Ephedra sinica* inhibit lipopolysaccharide-induced inflammatory mediators by suppressing nuclear factor -κB activation in RAW 264.7 macrophages. *Int. Immunopharmacol.* **2010**, *10*, 1616–1625. [CrossRef] [PubMed]

40. Kunert, O.; Swamy, R.C.; Kaiser, M.; Presser, A.; Buzzi, S.; Appa Rao, A.V.N.; Schühly, W. Antiplasmodial and leishmanicidal activity of biflavonoids from Indian *Selaginella bryopteris*. *Phytochem. Lett.* **2008**, *1*, 171–174. [CrossRef]
41. Mu, W.; Cheng, X.; Zhang, X.; Liu, Y.; Lv, Q.; Liu, G.; Zhang, J.; Li, X. Hinokiflavone induces apoptosis via activating mitochondrial ROS/JNK/caspase pathway and inhibiting NF -κB activity in hepatocellular carcinoma. *J. Cell. Mol. Med.* **2020**, *24*, 8151–8165. [CrossRef] [PubMed]
42. Shim, S.-Y.; Lee, S.; Lee, M. Biflavonoids isolated from *Selaginella tamariscina* and their anti-inflammatory activities via ERK1/2 signaling. *Molecules* **2018**, *23*, 926. [CrossRef] [PubMed]
43. Stiboller, M.; Freitas, F.P.; Francesconi, K.A.; Schwerdtle, T.; Nogueira, A.J.A.; Raber, G. Lipid-soluble arsenic species identified in the brain of the marine fish skipjack tuna (*Katsuwonus pelamis*) using a sequential extraction and HPLC/mass spectrometry. *J. Anal. At. Spectrom.* **2019**, *34*, 2440–2450. [CrossRef]
44. Taleshi, M.S.; Seidler-Egdal, R.K.; Jensen, K.B.; Schwerdtle, T.; Francesconi, K.A. Synthesis and characterization of arsenolipids: Naturally occurring arsenic compounds in fish and algae. *Organometallics* **2014**, *33*, 1397–1403. [CrossRef]
45. Zheng, Y.; Tian, C.; Dong, L.; Tian, L.; Glabonjat, R.A.; Xiong, C. Effect of arsenic-containing hydrocarbon on the long-term potentiation at Schaffer Collateral-CA1 synapses from infantile male rat. *Neurotoxicology* **2021**, *84*, 198–207. [CrossRef]
46. Kumar, A.; Basu, S.; Rishu, A.K.; Kumar, G. Revisiting the mechanisms of arsenic uptake, transport and detoxification in plants. *Environ. Exp. Bot.* **2022**, *194*, 104730. [CrossRef]
47. Snodgrass, W.R. Heavy metal toxicology. *Encycl. Food Health* **2016**, 328–331.
48. Besednova, N.N.; Zaporozhets, T.S.; Andryukov, B.G.; Kryzhanovsky, S.P.; Ermakova, S.P.; Kuznetsova, T.A.; Voronova, A.N.; Shchelkanov, M.Y. Antiparasitic effects of sulfated polysaccharides from marine hydrobionts. *Mar. Drugs* **2021**, *19*, 637. [CrossRef] [PubMed]
49. Gopalakrishnan, A.; Rajkumar, M.; Sun, J.; Parida, A.; Venmathi Maran, B.A. Integrated biological control of the weed water hyacinth using the novel method of grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) and the weevil *Neochetina* spp. *Chin. J. Oceanol. Limnol.* **2011**, *29*, 162–166. [CrossRef]