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Antiparasitic activity of the medicinal plant *Dillenia suffruticosa* against the marine leech *Zeylanicobdella arugamensis* (Hirudinea) and its phytochemical composition

Muhammad Dawood Shah[1] | Balu Alagar Venmathi Maran[1] | Mohammad Iqbal[2] | Fui Fui Ching[3] | Mohammad Tamrin Mohamad Lal[3] | Rafidah Binti Othman[3] | Rossita Shapawi[3]

[1]Endangered Marine Species Research Unit (UEMS), Borneo Marine Research Institute, University Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

[2]Biotechnology Research Institute, University Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

[3]Borneo Marine Research Institute, University Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

Correspondence
Balu Alagar Venmathi Maran, Endangered Marine Species Research Unit (UEMS), Borneo Marine Research Institute, University Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.
Email: bavmaran@ums.edu.my

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Abstract

Zeylanicobdella arugamensis (Annelida: Hirudinea), a marine parasitic leech, is currently affecting different species of cultured groupers, hybrid groupers, snappers and sea bass in Malaysia. *Dillenia suffruticosa* (Dilleniaceae), a medicinal plant found in Sabah, has been selected in our experiment to kill the leeches as a natural control method. The leech-infested hybrid groupers were collected from aquaculture facilities, and the isolated leeches were challenged against methanol extract of *D. suffruticosa* leaves. The experiment was carried out using various concentrations of the extracts such as 25, 50 and 100 mg/ml. The methanol extract showed significant antiparasitic activity against *Z. arugamensis* with 100% mortality at a concentration of 100 mg/ml in 14.39 ± 3.75 min., followed by 50 and 25 mg/ml in 32.97 ± 9.29 and 41.77 ± 5.40 min., respectively. The phytochemical composition of the extract was determined using GC-MS analysis to understand the nature of the principal compounds responsible for its antiparasitic properties. The leaves of *D. suffruticosa* demonstrated the presence of different bioactive compounds of various natures with varying percentages. Thus, it could be revealed that the methanol extract of *D. suffruticosa* mainly contains vital phytochemical compounds and showed an effective antiparasitic activity against the harmful leeches of hybrid groupers.

KEYWORDS

antiparasitic activity, aquaculture, *Dillenia suffruticosa*, *Epinephelus* spp., hybrid grouper, phytochemicals, *Zeylanicobdella arugamensis*

1 | INTRODUCTION

Zeylanicobdella arugamensis de Silva, 1963 is a marine ectoparasitic leech of the family Piscicolidae (Annelida: Hirudinea), affecting several cultured groupers and a wide range of other cultured fish species in Malaysia and neighbouring countries (Cruz-Lacierda, Toledo, Tan-Fermin, & Burreson, 2000; Kua, Choong, & Leaw, 2014; Ravi & Yahaya, 2017). The mortality of the host usually takes place due to the severe infestation of leeches within a period of three days followed by the secondary infections with different pathogenic bacteria (Kua et al., 2014). Usually, they attach in large numbers on the fins, jaw, inside the mouth and eyes of the fish (Cruz-Lacierda et al.,

2000). Due to these reasons, *Z. arugamensis* has been considered as an important threat to the aquaculture industry in South-East Asian countries (Ravi & Yahaya, 2017). The parasitic leech was controlled by exposing the infested fish to formalin (37% aqueous solution of formaldehyde) solution with various percentages (Cruz-Lacierda et al., 2000). Formalin bath is very toxic for fish and human health; and it removes oxygen from the aquatic environment (National Toxicology Program, 2011; Pitten, Kramer, Herrmann, Bremer, & Koch, 2000; Plumb, 1992). In addition, formaldehyde is a carcinogenic agent and exerts neurotoxic effects on fish as well as other animals and human (National Toxicology Program, 2011).

Dillenia suffruticosa is a medicinal plant and belonging to the family Dilleniaceae (Corner, 1997). The local Malay name used for the plant is 'Simpoh air' or 'Simpoh ayer' (Yussof, Ahmad, & Pasok, 2010). The plant has been reported from various parts of Asia such as Malaysia, Singapore, Brunei, Indonesia and Sri Lanka. The plant grows on eroded soil, wasteland, forest edges and swampy areas (Corner, 1997; Foo, 1985). This medicinal plant has been used in different parts of Malaysia for the treatment of stomach pain, fever and cleaning wounds (Yussof et al., 2010). It has also reported with antifungal, antibacterial and antiviral properties (Muliawan, 2008). However, there is so far no report on the antiparasitic activity of *D. suffruticosa* against any parasites. The main objectives of the present study were to evaluate the antiparasitic activity and phytochemical composition of the methanol extract of *D. suffruticosa*.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Formalin (37% aqueous formaldehyde solution) purchased from Sigma, Leica, Microsystem, and Germany. HPLC grade methanol was obtained from Merck.

2.2 | Plant collection

The leaves of the plant were collected from Papar (5.7346°N, 115.9319°E), Kota Kinabalu, Sabah, East Malaysia. The identification of the plant was carried out, and a voucher specimen (MDS-002) was deposited at the Institute for Tropical Biology and Conservation, University Malaysia Sabah, Kota Kinabalu.

2.3 | Extraction

The mature leaves were rinsed with distilled water and dried in an oven at 37°C. The dried plant leaves were ground separately in a mechanical grinder and stored in an airtight container. The powdered leaves (60 g) of the plant was extracted with HPLC grade methanol (300 ml) using soxhlet method (50–60°C for 72 hr). The methanol residues were removed from the extract using a vacuum rotary evaporator. The samples were kept at −80°C for 24 hr and then lyophilized using a freeze drier. The freeze-dried samples were then stored in the freezer for further studies (Osadebe et al., 2012).

2.4 | Gas chromatography–mass spectrometry (GC-MS) analysis of *Dillenia suffruticosa*

Prepared samples of *D. suffruticosa* were injected into a GC-MS system consisting of an Agilent 7890A gas chromatograph system coupled with an Agilent 5975C mass spectrometry detector. A capillary column HP-5MS (30 m × 0.25 mm) of 0.25 µm film thickness of coated material was used. The injector temperature was set at 250°C, and the temperature settings were as follows: start at 40°C and hold for 3 min; from 40 to 200°C at 3°C/min. and then hold for 3 min. A post-run of 5 min. at 200°C was adjusted for the next injection. Pure helium gas was used as a carrier gas and maintained at a constant flow rate of 1.0 ml/min. Gas chromatography was done in splitless mode. Identification of compounds was done by referring to the National Institute of Standards and Technology (NIST) library, and the compositions were computed with reference to the abundance of the compounds in the chromatogram. Each analysis was performed in triplicates.

2.5 | Antiparasitic bioassay

Ectoparasites were collected from the infested hybrid groupers of TGxGG (*Epinephelus fuscoguttatus*×*E. lanceolatus*) (Figure 2) from the hatchery of University Malaysia Sabah. All parasites were well maintained under standard ethical principles as per university regulations (UMS/IP7.5/M3/4-2012). The collected parasites were identified based on their morphological features. A total of 120 adult parasites were selected, divided into 5 groups, and each group was provided with 4 leeches in a Petri dish. Group 1 served as a negative control, treated with sea water; group 2 as a positive control, treated with 0.25% (v/v) formalin solution, while groups 3, 4 and 5 were challenged with 25, 50 and 100 mg/ml of the methanol extract of *D. suffruticosa* respectively. During the challenge, the behaviour of parasites was observed keenly and recorded. Parasites inactivity and death were recorded using the stopwatch. The experiment was repeated for six times in six replicates.

2.6 | Statistical analysis

The data were analysed using SPSS 21.0 windows statistical package software (SPSS Inc). Significant differences between groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. All results were presented as mean ± standard deviation of the mean (S.D.). p Values <0.05 were regarded as significant.

3 | RESULTS

3.1 | Phytochemical analysis by GC-MS

Gas chromatography and mass spectrometry analyses of constituents were carried out in the methanolic extract of *D. suffruticosa*. Figure 1 indicates the obtained chromatogram. Various peaks indicated in the chromatogram confirm the detection of different

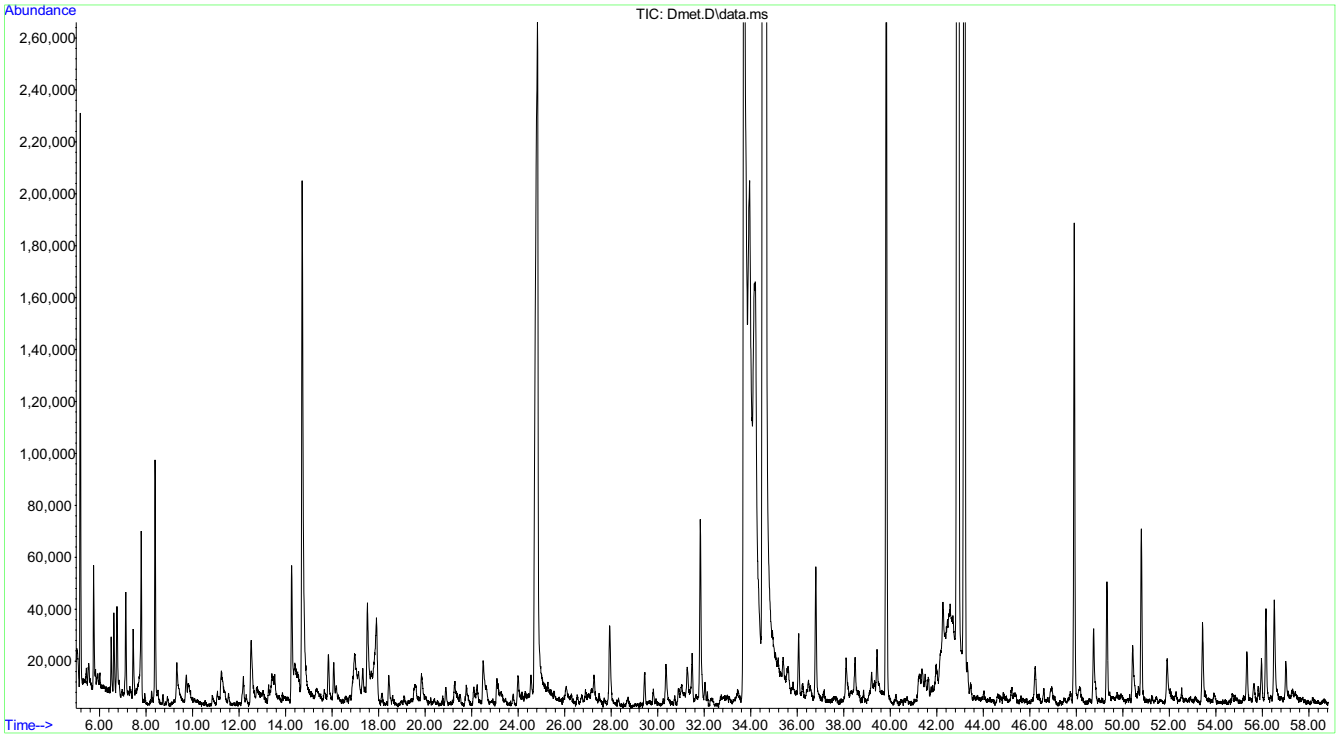

FIGURE 1 GC-MS chromatogram of the methanol extract of *Dillenia suffruticosa* [Colour figure can be viewed at wileyonlinelibrary.com]

phytochemical compounds at various retention times. Nineteen phytochemical compounds of different functional groups such as phenolic, carboxylic, aromatic diterpene, triterpene, saturated and unsaturated fatty acid groups were tabulated along with their area percentage and retention times (Table 1).

3.2 | Antiparasitic activity of *Dillenia suffruticosa*

The severely infested ectoparasites of hybrid groupers were identified as *Zeylanicobdella arugamensis* de Silva, 1963, belonging to the family Piscicolidae (Annelida: Hirudinea) (Figure 2). The mortality time of the leeches treated in formalin and extract treated groups are given in Table 2. The plant treated groups were showing the effect in a dose-dependent manner. The time taken for the mortality of leech is lesser (14.39 ± 3.75) in 100 mg/ml dose than 25 mg/ml (41.77 ± 5.40) of the methanol extract of *D. suffruticosa* (Table 2).

3.3 | The behaviour of *Zeylanicobdella arugamensis*

The leech *Z. arugamensis* use their two suckers for locomotion and attachment. They attach either on the fish or on the fish tank using the larger posterior sucker, then extend their body and attach again using the small anterior oral sucker. The posterior sucker then detached, drawn forward and reattached near the fixed anterior sucker, which is then released. This locomotion is well organized and rapid. It also swims fast enough to reach and find the attachment area. After detachment, the posterior sucker is applied high up on the ventral surface making loop shape. When the parasites were exposed to various concentration of methanol extract, they sway in random movements and were not able to attach using either posterior or anterior suckers to the bottom of the Petri dish. Gradually, they became weak and die. The bodies of leeches became wrinkled and were floating in the methanol extract solution. While in the case of formalin, the leeches could be able to attach using the posterior sucker to the bottom of the Petri dish before they were dead. In the control, they attach firmly on Petri dish using its suckers and locomotion was observed.

3.4 | Physico-chemical parameters

Table 3 showed the water quality parameters in the control and treated groups. All parameters determined were remained constant except with a change in pH and dissolved oxygen of the plant treated groups as compared to the normal control group. Dissolved oxygen was 6.4 mg/L, while pH ranged from 4.25 to 4.66 in the plant treatment group as presented in Table 3. The change in pH of the plant solution might be due to the presence of different bioactive compounds with acidic nature.

4 | DISCUSSION

The leech *Z. arugamensis* is the most harmful parasite in the aquaculture facilities of groupers in South-East Asian countries (Cruz-Lacierda et al., 2000; Ravi & Yahaya, 2017). In order to control the leeches, fish farmers employed the chemical method by using formalin, which is against the eco-friendly aquaculture and freshwater bath as well (personal observation). On the other hand,

TABLE 1 Phytochemical compounds detected in the methanol extract of *Dillenia suffruticosa* (RT = retention time)

No	Compounds	RT	Area (%)
1.	Methyl Glycolate	4.25	2.95
2.	2-Furanmethanol	7.44	0.77
3.	Phenol	12.52	1.39
4.	Benzyl Alcohol	14.26	2.04
5.	Undecane	17.09	11.97
6.	2h-Pyran−2-One, 4,6-Dimethyl-	22.5	1.19
7.	1-Undecanol	30.35	0.78
8.	Tetradecane	31.48	0.62
9.	Tridecanal	31.83	0.03
10.	Cyclododecane	33.71	35.78
11.	1-Dodecene	34.19	19.46
12.	Phenol, 2,4-Bis(1,1-Dimethylethyl)	36.06	0.83
13.	*n*-Tridecan−1-ol	36.8	1.98
14.	Dodecanoic acid	38.01	0.84
15.	Lauryl acetate	39.83	12.26
16.	3-Chloropropionic acid, heptadecyl	42.57	1.28
17.	Hexadecanoic acid, methyl ester	50.79	2.66
18.	*n*-Hexadecanoic acid	51.9	0.96
19.	Phytol	56.51	2.13

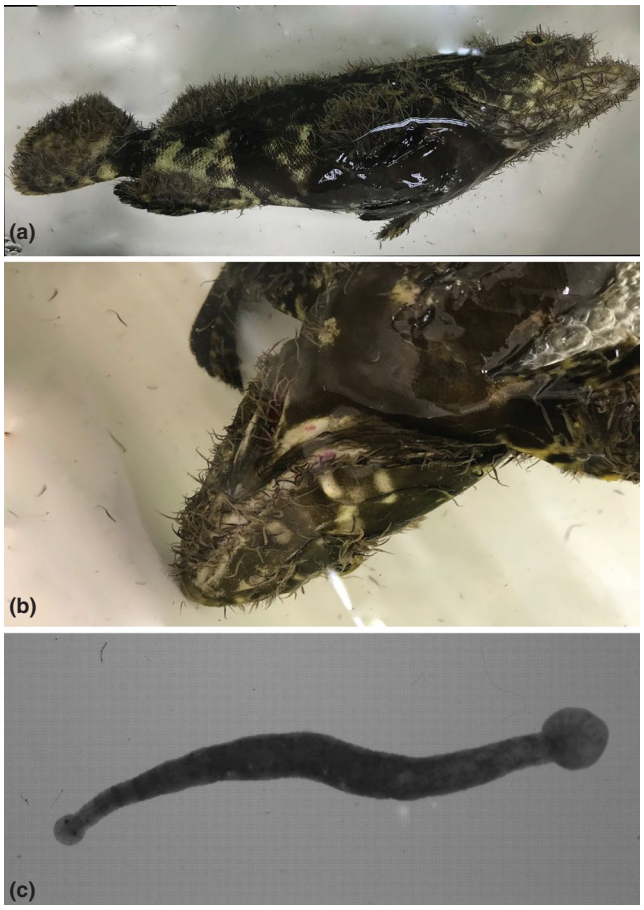

FIGURE 2 (a) Hybrid grouper (TGGG) infested with leech *Zeylanicobdella arugamensis*; (b) showing mouth area with infestation; (c) Habitus of adult *Zeylanicobdella arugamensis* de Silva, 1963 (Annelida: Hirudinea: Piscicolidae) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Mortality time of parasites at different concentrations of methanol extract of *Dillenia suffruticosa*

No	Group	Mortality time (min) Mean ± S.D
1	Negative control	0.00 ± 0.00
2	Positive control (Formalin 0.25% (v/v))	3.99 ± 0.93[a]
3	*D. suffruticosa* (25 mg/ml)	41.77 ± 5.40[a,b]
4	*D. suffruticosa* (50 mg/ml)	32.97 ± 9.29[a,b,c]
5	*D. suffruticosa* (100 mg/ml)	14.39 ± 3.75[a,b,c,d]

Note: Each value represents mean ± *SD* of four parasites.
[a]Significance at p = 0.05 compared with the control group.
[b]Significance at p = 0.05 compared with the formalin treated group (0.25% [v/v]).
[c]Significance at p = 0.05 compared with *D. suffruticosa* (25 mg/ml).
[d]Significance at p = 0.05 compared with *D. suffruticosa* (50 mg/ml).

the medicinal plant extracts can be used as a bio-safe and natural remedy for the parasite infestations due to the presence of various bioactive compounds with antioxidant properties (Rafieian-Kopaei, 2012).

In this study, we experimented with the medicinal plant *D. suffruticosa* to kill marine leeches infesting severely on hybrid groupers (see Figure 2). Before the exposure of parasites to the methanol extracts of plants, the GC-MS analysis was done and showed the presence of various bioactive compounds such as benzyl alcohol, 2h-pyran-2-one, 4, 6-dimethyl, phenol, 2,4-bis(1,1-dimethylethyl) and dodecanoic acid (Lauric acid). These compounds have been reported with antiparasitic (Cunha, 2019; Rayan & McDonnell, 2014), antifungal (Mikhlin, Radina, Dmitrovskiĭ, Blinkova, & Butova, 1983), antioxidant, antimicrobial (Dasgupta & Humphrey, 1998; Lee & Shibamoto, 2002; Lee, Umano, Shibamoto, & Lee, 2005), anti-inflammatory, antiviral (Appendino et al., 2007) and anti-cancer properties (Ajayi, Olagunju, Ademuyiwa, & Martins, 2011; Rajaram et al., 2013). In addition to this saturated fatty acids, esters and a fatty acid such as hexadecanoic acid, ethyl ester and n-hexadecanoic acid were also found in the methanol extracts of *D. suffruticosa*. These compounds have also been detected in some other plant extracts (BülentKöse, Iscan, Demirci, Başer, & Celik, 2007; Kumar, Kumaravel, & Lalitha, 2010). Phytol is another diterpene alcohol bioactive compound detected in the methanol extract of *D. suffruticosa*, and significant antiparasitic activity has been reported (De Moraes et al., 2014). The compound has also been found with antimicrobial and anti-cancer properties (Kumar et al., 2010).

TABLE 3 Water quality parameters of control and treatment groups

Water quality parameters	Concentrations				
	Negative Control	Positive Control Formalin (0.25%) (v/v)	*Dillenia suffruticosa* (mg/ml)		
			25	50	100
Temperature (°C)	24.9	22.6	23.3	22.7	23.0
pH	7.89	7.24	4.66	4.44	4.25
Salinity (ppt)	30.9	30.9	30.9	30.9	30.9
Dissolved oxygen (mg/L)	4.23	6.30	6.40	6.40	6.40

The exposure of various concentrations of the plant extract resulted in 100% of mortality in a dose-dependent manner at different times (Table 2). Leech parasites showed 100% mortality at a concentration of 100 mg/ml (*D. suffruticosa*) in <15 min, followed by 50 mg/ml and 25 mg/ml in 32.97 and 41.77 min, respectively, compared to the normal control group treated with sea water. The group treated with 0.25% (v/v) of formalin also resulted in 100% mortality of *Z. arugamensis* in 3.99 min. Similar results of 80% and 100% mortalities' were reported by Rizky, Cheng, and Nursyam, (2018) by exposing *Piscicola geometra* leech parasite to *Scutellaria baicalensis* and *Morinda citrifolia* plant extracts. The methanol extract of the plant *Vitis vinifera* (300 mg/L and 600 mg/L) against the leech *Limnatis nilotica* resulted in the mortality of the parasite in 15.4 and 10.1 min respectively (Gholami-Ahangaran, Bahmani, & Zia-Jahromi, 2012). Likewise, the methanolic extract of *Allium sativum* demonstrated anti-leech activities with the average killing time of 68.44 ± 28.39 min (Bahmani, Abbasi, Mohsenzadegan, Sadeghian, & Ahangaran, 2011). The natural agents such as caprylic acid, peppermint oil and cinnamon oil had efficacy against the larvae of dictidophorid monogenean *Heterobothrium okamotoi* infesting the tiger puffer in Japan (Hirazawa, Ohtaka, & Hata, 2000). In another study, caprylic acid at the concentration of 1 mM had an antiparasitic effect against several other fish parasites such as the ciliate *Cryptocaryon irritans* theronts, monogenean *Benedenia seriolae* oncomiracidia and myxosporean *Kudoa shiomitsui* spores, but not against the sea lice *Pseudocaligus fugu* (=*Caligus fugu*) copepodids (Hirazawa, Oshima, & Hata, 2001).

In this study, we used formalin as a positive control because it is used by local farmers for the antiparasitic activity with a low concentration ranging from 0.1% to 1%. The group treated with 0.25% (v/v) of formalin also resulted in 100% mortality of *Z. arugamensis* in 3.99 min. The chemical is not only toxic to fish but also to human beings (National Toxicology Program, 2011; Pitten et al., 2000; Plumb, 1992). A large number of chemicals and drugs are used in the aquaculture industry globally but only a few have been specifically developed for the aquaculture industry per se (Pitten et al., 2000). The majority are holdovers from wastewater treatment or veterinary and human drugs (Mohamed, Nagaraj, Chua, & Wang, 2000). Thus, much of chemicals and drugs used are based on ease of availability and on guesswork, rather than on a methodical protocol linking identifying pathogens and prescribing the appropriate administration (Bahmani, Hosseini, Avijgan, & Qorbani, 2010). Hitherto, in Malaysia and Singapore large number of chemotherapeutant (chemicals and drugs) are used as disinfectants in aquaculture facilities in order to increase fish production. The most important of these chemicals and drugs are formalin, benzalkonium chloride, acriflavine, malachite green, hypochlorite, polyvinyl pyrrolidine, sulphonamides, tetracyclines, nitrofurans, chloramphenicol, oxolinic acid, and virginiamycin (Bahmani, et al., 2010; Bahmani, Rafieian-kopaei, Parsaei, & Mohsenzadegan, 2012; Mohamed et al., 2000; Morrison, Fox, & Rogers, 2011). The known side effects of some of these chemicals and drugs are oxygen depletion, destroying beneficial bacteria such as the nitrifying bacteria, and carcinogenic (Herwig, 1979; Mohamed et al., 2000; Plumb, 1992).

The plant extract also induces some behavioural changes in the leech parasite as compared to the normal control group. The locomotion was disturbed, and the parasites were not able to attach using the suckers to the bottom of the Petri dish due to the presence of various bioactive compounds with antiparasitic properties. In sea water, freely leeches attached to the bottom by the posterior sucker, then extended its body and used the oral sucker for the re-attachment (Kearn, 2004). However, in formalin, only one sucker either anterior or posterior attaches and then no movement was observed; under the plant extracts, both suckers were not active and just fell down in this experiment. Since *Z. arugamensis* is reported from Malaysia, Singapore, Sri Lanka, the Philippines, Indonesia and India (Cruz-Lacierda et al., 2000; De Silva, 1963; De Silva & Fernando, 1965; Sanjeeva Raj, Jayadev, & Gladstone, 1977), this study is imperative to control the infestation of leeches in aquaculture facilities.

5 | CONCLUSION

The data of the current investigation clearly shows that the methanol extract of the plant *Dillenia suffruticosa* is a good source of bioactive compounds of various natures in various percentages. The treatment indicated that methanol extract of *D. suffruticosa* showed strong antiparasitic activity with 100% mortality against the marine leech *Z. arugamensis*. Further study on the exposure of infested groupers with the methanolic extract of *D. suffruticosa* will be required in the future.

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CONFLICT OF INTEREST

The authors disclose that there are no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

MDS, BAVM and MI involved in conceptualization; investigation; methodology; data curation; and formal analysis. BAVM, CFF, MTML, RO and RS involved in funding acquisition and project administration. MDS and BAVM wrote the original draft; BAVM, MI, CFF, MTML, RO and RS reviewed and edited.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ORCID

Balu Alagar Venmathi Maran https://orcid.org/0000-0003-1090-5295

Rossita Shapawi https://orcid.org/0000-0003-1548-3327

REFERENCES

Ajayi, G. O., Olagunju, J. A., Ademuyiwa, O., & Martins, O. C. (2011). Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn. *Journal of Medicinal Plants Research*, *5*, 1756–1761.

Appendino, G., Ottino, M., Marquez, N., Bianchi, F., Giana, A., Ballero, M., & Munoz, E. (2007). Arzanol, an anti-inflammatory and anti-HIV-1 phloroglucinol a-pyrone from *Helichrysum italicumssp*, microphyllum. *Journal of Natural Product*, *70*, 608–612.

Bahmani, M., Abbasi, J., Mohsenzadegan, A., Sadeghian, S., & Ahangaran, M. G. (2011). *Allium sativum L.*: The anti-immature leech (*Limnatis nilotica*) activity compared to Niclosomide. *Comparative Clinical Pathology*, *22*, 1380–1387.

Bahmani, M., Hosseini, S. R., Avijgan, M., & Qorbani, M. (2010). Evaluating the anti-leech effects of tobacco methanolic extract compared with succinyle choline and some other anti-parasite drugs. *Journal of Shahrekord University of Medical Sciences*, *12*, 53–59.

Bahmani, M., Rafieian-kopaei, M., Parsaei, P., & Mohsenzadegan, A. (2012). The anti-leech effect of *Peganum harmala* L. extract and some antiparasitic drugs on *Limnatis nilotica*. *African Journal of Microbiology Research*, *6*, 2586–2590.

BülentKöse, Y., Iscan, G., Demirci, B., Başer, K. H., & Celik, S. (2007). Antimicrobial activity of the essential oil of *Centaurea aladagensis*. *Fitoterapia*, *78*, 253–254. https://doi.org/10.1016/j.fitote.2006.12.002

Corner, E. J. H. (1997). *Wayside trees of Malaya* (4th ed.). Selongor, Malasia: Malayan Nature Society.

Cruz-Lacierda, E. R., Toledo, J. D., Tan-Fermin, J. D., & Burreson, E. M. (2000). Marine leech (*Zeylanicobdella arugamensis*) infestation on cultured orange-spotted grouper, *Epinephelus coioides*. *Aquaculture*, *185*, 191–196.

Cunha, J. P. (2019). August 01. *Benzyl alcohol*. Medical and pharmacy editor. Retrieved from https://www.rxlist.com/consumer_benzyl_alcohol_ulesfia/drugs-condition.htm

Dasgupta, A., & Humphrey, P. E. (1998). Gas chromatographic–mass spectrometric identification and quantitation of benzyl alcohol in serum after derivatization with perfluorooctanoyl chloride: A new derivative. *Journal of Chromatography B: Biomedical Sciences and Applications*, *708*, 299–303. https://doi.org/10.1016/S0378-4347(97)00679-8

De Moraes, J., de Oliveira, R. N., Costa, J. P., Junior, A. L. G., de Sousa, D. P., Freitas, R. M., & Pinto, P. L. S. (2014). Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease Schistosomiasis mansoni. *PLoS Neglected Tropical Diseases*, *8*, e2617. https://doi.org/10.1371/journal.pntd.0002617

De Silva, P. H. D. H. (1963). *Zeylanicobdella arugamensis gen. nov. and sp. nov. from Arugam Kalapu, Eastern Province, Ceylon*. Spolia Zeylanica, *30*, 47–53.

De Silva, P. H. D. H., & Fernando, C. H. (1965). *Three marine leeches (Piscicolidae, Hirudinea) from the Malay Peninsula*. Spolia Zeylanica, *30*, 227–232.

Foo, T. S. (1985). *A Guide to the wild flowers of Singapore*. Singapore Science Center, 24.

Gholami-Ahangaran, M., Bahmani, M., & Zia-Jahromi, N. (2012). Comparing of anti-leech effect of *Vitis vinifera* L. Plant and Niclosamide on different forms of *Limnatis nilotica*. *World Applied Science Journals*, *20*, 1082–1085.

Herwig, N. (1979). *Handbook of drugs and chemicals used in treatment of fish diseases*. Charles C. Thomas, Springfield, IL.

Hirazawa, N., Ohtaka, T., & Hata, K. (2000). Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*. *Aquaculture*, *188*, 1–13. https://doi.org/10.1016/S0044-8486(00)00334-3

Hirazawa, N., Oshima, S., & Hata, K. (2001). In vitro assessment of the antiparasitic effect of caprylic acid against several fish parasites. *Aquaculture*, *200*, 251–258. https://doi.org/10.1016/S0044-8486(01)00529-4

Kearn, G. C. (2004). *Leeches, lice and lampreys. A natural history of skin and gill parasites of fishes* (p. 432). The Netherlands: Springer.

Kua, B. C., Choong, F. C., & Leaw, Y. Y. (2014). Effect of salinity and temperature on marine parasite, *Zeylanicobdella arugamensis* De Silva, under laboratory conditions. *Journal of Fish Disease*, *37*, 201–207.

Kumar, P. P., Kumaravel, S., & Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *Africa Journal of Biochemical Research*, *4*, 191–195.

Lee, K. G., & Shibamoto, T. (2002). Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *Journal of Agriculture and Food Chemistry*, *50*, 4947–4952. https://doi.org/10.1021/jf0255681

Lee, S. J., Umano, K., Shibamoto, T., & Lee, K. G. (2005). Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. *Food Chemistry*, *91*, 131–137. https://doi.org/10.1016/j.foodchem.2004.05.056

Mikhlin, E. D., Radina, V. P., Dmitrovskiĭ, A. A., Blinkova, L. P., & Butova, L. G. (1983). Antifungal and antimicrobial activity of beta-ionone and vitamin A derivatives. *Prikl Biokhimia Dan Mikrobiologi*, *19*(6), 795–803.

Mohamed, S., Nagaraj, G., Chua, F. H. C., & Wang, Y. G. (2000). *The use of chemicals in aquaculture in Malaysia and Singapore*, In: J.R. Arthur, C.R. Lavilla-Pitogo, pp. 191–196.

Morrison, J. R., Fox, S. R., & Rogers, W. A. (2011). Control of an infestation of a fish leech (*Myzobdella lugubris*) on catfishes in tanks and earthen ponds. *Journal of Aquatic Animal Health*, *5*, 110–114.

Muliawan, S. Y. (2008). *Effect of* Dillenia suffruticosa *extract on dengue virus type 2 replication*. Universa Medicina, *27*, 1–5.

National Toxicology Program (2011). *NTP 12th Report on Carcinogens*. Rep. Carcinog., *12*, iii–499.

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 Disease*, *5*, 289–293. https://doi.org/10.1016/S1995-7645(12)60041-8

Pitten, F. A., Kramer, A., Herrmann, K., Bremer, J., & Koch, S. (2000). Formaldehyde neurotoxicity in animal experiments. *Pathology Research and Practice*, *196*, 193–198. https://doi.org/10.1016/S0344-0338(00)80100-4

Plumb, J. A. (1992). Disease control in aquaculture. In M. Shariff, R. P. Subasinghe, & J. R. Arthur (Eds.), *Diseases in Asian aquaculture. Fish health section* (pp. 3–17). Manila: Asian Fisheries Society.

Rafieian-Kopaei, M. (2012). Medicinal plants and the human needs. *Journal of Herbmed Pharmacology*, *1*, 1–2.

Rajaram, K., Moushmi, M., Velayutham Dass Prakash, M., Kumpati, P., Ganasaraswathi, M., & Sureshkumar, P. (2013). Comparative bioactive studies between wild plant and callus culture of *Tephrosia tinctoria* pers. *Applied Biochemistry and Biotechnology*, *171*, 2105–2020. https://doi.org/10.1007/s12010-013-0444-3

Ravi, R., & Yahaya, S. Z. (2017). *Zeylanicobdella arugamensis*, the marine leech from cultured crimson snapper (*Lutjanus erythropterus*), Jerejak Island, Penang, Malaysia. *Asian Pacific Journal of Tropical Biomedicine*, *7*(5), 473–477. https://doi.org/10.1016/j.apjtb.2017.01.018

Rayan, P., & McDonnell, P. A. (2014). Dietary supplementation of dodecanoic acid in maternal mice enhances resistance to *Giardia duodenalis* infection in suckling neonatal pups. *Pharmacognosy Communications*, *4*, 84–89.

Rizky, P. N., Cheng, T. C., & Nursyam, H. (2018). Anti-parasite activity of *Scutellaria baicalensis* and *Morinda citrifolia* extracts against *Piscicola geometra*. *Earth Environmental Science*, *137*, 01203.

Sanjeeva Raj, P. J., Jayadev, B. S. & Gladstone, M. (1977). Anatomical details of two fish parasites from the Pulicat Lake, South India. *Journal of the Marine Biological Association of India*, *19*, 35–43.

Yussof, M. M., Ahmad, B., & Pasok, G. (2010). *Traditional medicinal plants of the Dusun Tobilung of Kampong Toburon, Kudat, Sabah, Malaysia*. (Research Notes): An article from: Borneo Research Bulletin. Borneo Research Council Inc., Philipes, ME., USA.

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