Antiparasitic Potential of a Medicinal Plant Flower against Marine Parasitic Leech in Aquaculture

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ABSTRACT The aquaculture industry is essential for food production and economic development in Malaysia and other parts of the world. The development is affected by the parasitic infestation on different cultured fish species. The most common marine parasitic leech *Zeylanicobdella arugamensis* (Hirudinea), which infests various cultured fishes in Malaysia, also in Southeast Asian countries. The current research aimed to elucidate the methanol extract of the flower of *Dillenia suffruticosa* (DS) belonging to the family Dilleniaceae as a natural control agent against marine parasitic leech *Z. arugamensis*. The leeches were collected from infested hybrid groupers in aquaculture facilities and challenged with various concentrations (25, 50 and 100 mg/ml) of the methanol extract of DS flower. The results demonstrated significant antileech activity against *Z.arugamensis* with total mortality in an average period of 53.77 ± 1.42, 37.63 ± 5.35 and 7.51 ± 0.74 min, respectively. Thus, the research displayed that the methanol extract of DS flower can act as a natural control agent against marine parasitic leech. The study will benefit fish farmers in Malaysia and other Southeast Asian countries to control the leeches using natural products.

KEYWORDS: Aquaculture, Antileech activity, Hybrid Grouper, *Zeylanicobdella arugamensis*, Dillenia, Flower, Natural control agent.

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INTRODUCTION

The role of aquaculture in food production and economic development in Malaysia and other countries is vital. The development of aquaculture is affected by the spread of diseases and parasitic infestation (Venmathi Maran *et al*., 2009; Fathi *et al*., 2018). The parasitic infestation not only results in weakness and infections but also cause mortality of the cultured species which lead to food shortage and huge economical losses (Barber *et al*., 2000; Venmathi Maran *et al*., 2012; Shinn *et al*., 2015; Venmathi Maran *et al*., 2016). One of the common parasites affecting various families of cultured fishes including grouper, seabass, snapper, etc., is *Zeylanicobdella arugamensis* (Hirudinea), a marine leech attached to the mouth, fins, gills and tail of the host fish, sucks blood and cause mortality of the host (Cruz-Lacierda *et al*., 2000).

In Malaysia, various harmful chemicals are applied in aquaculture facilities for the control of parasites. The commonly used chemicals are organophosphate, copper sulphate, formalin, malachite green etc. These chemicals are extremely harmful to the fish, farmers and surrounding environment (Shariff *et al*., 2000; Leal *et al*., 2018). To avoid the consumption of toxic chemicals in the aquaculture industries the development of safe and cheap biocontrol agents is the demand of the day. The current research aimed to select the methanol extract of the *Dillenia suffruticosa* (DS) (Dilleniaceae) flower as a natural control agent against marine parasitic leech *Zeylanicobdella arugamensis*.

METHODOLOGY

Flower Collection

Dillenia suffruticosa flowers were collected from Universiti Malaysia Sabah, Kota Kinabalu (5.7346° N, 115.9319° E), Sabah between 6 and 7 January 2020 during a sunny day (24 - 33 °C). The fully grown flowers were yellow and 6 to 10 cm wide. The identification of the plant was followed by Hooker and Thomson (1872).

Solvent Extraction

The DS flowers were rinsed with distilled water and dried in the oven at 37^{0}C. The dried flowers were grounded in a heavy-duty grinder and kept in an airtight container. Around 50g of powder was extracted with HPLC grade methanol (250ml) using the maceration method. The methanol residues were removed from the extract using a vacuum rotary evaporator. The samples were kept at -80 ^{0}C for 24 h and then lyophilized using a freeze drier. The freeze-dried samples were then stored in the freezer for further analysis.

Antileech Bioassay

Parasitic leeches (*Z. arugamensis*) were obtained from the infested hybrid groupers (*Epinephelus fuscoguttatus* x *E.lanceolatus*) from the aquaculture facilities of Universiti Malaysia Sabah (Figure 1). Mature parasites (1.1-1.4 cm in size) were divided into 5 groups (6 leeches per group), negative control (group 1), positive control (group 2, treated with 0.25% formalin solution), extract treatment (groups 3, 4 and 5 administered with 25, 50 and 100 mg/ml of the methanol extract DS flowers. During the challenge, leech inactivity and mortality were noted (Shah *et al*., 2020a, Venmathi Maran *et al*., 2021).

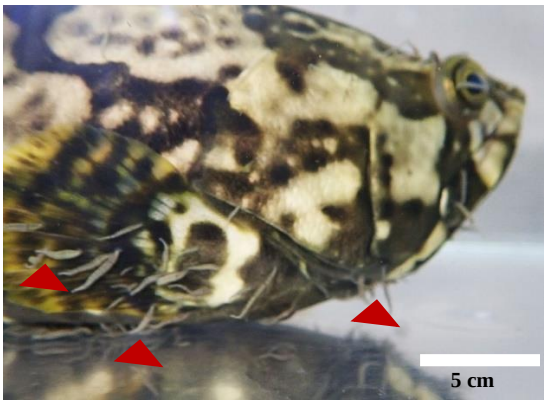

Figure 1. *Zeylanicobdella arugamensis* infested hybrid grouper *(Epinephelus fuscoguttatus* x *E.lanceolatus)*

RESULTS AND DISCUSSION

The effect of the methanol extract of DS flower is indicated in Table 1. Total leech mortality was obtained by the administration of the methanol extract of the DS flower. The water quality parameters of the normal control, positive control and extract treated solutions are shown in Table 2. All the parameters remain constant while slight changes were noticed in the pH of the extract solution in comparison with normal and positive control.

Table 1: Mortality time of parasitic leeches at different concentrations of *Dillenia suffruticosa* flower (DS) methanol extract

No	Group	Mortality Time (min) (Mean ± SD)	Percentage Mortality
1	Normal control	120 ± 00	0
2	Positive control (Formalin 0.25%) (v/v)	3.63 ± 0.40 [#]	100
3	DS flower (25 mg/ml)	53.77 ± 1.42 [#,&]	100
4	DS flower (50 mg/ml	37.63 ± 5.35 [#,&,$]	100
5	DS flower (100 mg/ml)	7.51 ± 0.74 [#,&,$,*]	100

Each value represents the mean±SD of 6 parasitic leeches per group.
[#]Significance at $p<0.05$ compared with the control group.
[&]Significance at $p<0.05$ compared with the formalin treated group (0.25% v/v)
[$]Significance at $p<0.05$ compared with DS flower (25 mg/ml)
[*]Significance at $p<0.05$ compared with DS flower (50 mg/ml)

Table 2: Water quality parameters of the solution for the treatment of parasitic leeches

No	Water parameters			Concentrations		
	Groups	Normal control	Positive control (formalin 0.25%) (v/v)	DS flower (mg/ml)		
				25	25	(25)
1	Temperature (^{0}C)	25.6	24.9	24.9	24.9	24.9
2	pH	7.77	7.30	4.16	4.60	5.10
3	Salinity (ppt)	30.0	30.9	30.0	30.0	30.9
4	Dissolved oxygen (mg/l)	7.1	6.6	6.8	7.1	7.1

The methanol extract of the DS flowers also induces behavioral changes in the parasitic leech (Figure 2). The leeches in the control group indicated usual behavior, attached firmly to the bottom of the petri dish via suctions and swimming in a zig-zag pattern. The leeches exposed to formalin chemical demonstrated extreme vigorous swimming for the first min and finally become weak and dead. The exposure of the leeches to the methanol extract of DS flower resulted in the active swimming initially. After 10 mins the leeches showed less movement, rolling their bodies and scattered from each other and after 20 min leeches become weak while after 35 mins, no movement was noticed. Finally, all the leeches were dead with curved bodies floating on the surface. The exposure of leeches to methanol extract of DS flower at a concentration of 50 mg/ml resulted in aggressive swimming with no attachment of suctions. After 10 mins they showed less movement, scattered from each other, rolling from one place to another while after 30 min no movements were noticed. At a concentration of 100 mg/ml, the leeches showed vigorous swimming for 1 min with no attachment of the suctions and after 5 min all the movements were stopped before they were dead.

Figure 2. Normal and flower treated leeches. A= normal leech, treated with seawater only, B = leech treated with 0.25 % of formalin solution, C= leech treated with methanol extract of DS flower.

Our results demonstrated that the methanol extract of DS flower resulted in the completed mortality of the parasitic leeches at various concentrations. All the leeches were dead in a limit of 53.77, 37.63 and 7.51 min at the concentration of 25, 50 and 100 mg/ml. In the study, formalin was also applied as a positive control. The chemical resulted in the completed mortality of the leeches in an average time of 3.63 min. The chemical is applied in the aquaculture industries for parasites control but it is toxic to fish, humans and the surrounding environment (Piper and Smith, 1973; Francis-Floyd, 1996; Pitten *et al*., 2000; Leal *et al*., 2018). As an alternative, the natural product can be applied as a biocontrol agent due to the presence of different bioactive compounds with zero or less side effects (Bahmani and Rafieian-Kopaei, 2014**).** Besides the antiparasitic potential, the natural product also plays an important role in the enhancement of growth performance by the activation of the intestinal digestive enzymatic levels and gene expression of the related genes to the liver, kidney brain and other vital tissues (Zemheri-Navruz *et al*., 2020). Compared to our data some other plants have also been reported with antiparasitic potential. The effectiveness of various chromatographic fractions of *D. suffruticosa* (Dilleniaceae) leaves methanol extract have been reported against the parasitic leech *Z. arugamensis* (Shah *et al*., 2020b). Fraction 6 of the plant methanol extract killed all the leeches in 31 min at a concentration of 20mg/ml (Shah *et al*., 2020b). The extract of *Scutellaria baicalensis* (Lamiaceae) was applied against the marine parasitic leech *Piscicola geometra* (Linnaeus, 1761) (Hirudinea) (Habtemariam, 2019; Raditic and Bartges, 2013; Rizky *et al*., 2018). The leeches were treated for 96h with different dilutions of plant extract (1/50x to 1/1000x). Completed mortality in a time limit of 8h, 40h, 48h, 72h, and 96h was reported (Rizky *et al*., 2018). From the reported data it is obvious that natural product has the antileech potential and the methanol extract of our sample resulted in the elimination of the parasitic leeches in a very short time of around 8 min at a higher concentration (100 mg/ml). Several extracts and chromatographic fractions of the Bornean herbal plant are effective against leech infestation (Shah *et al*., 2020c; Shah et al., 2021; Venmathi Maran *et al*., 2021). Thus, the methanol extract of the DS flower can be applied as a biocontrol agent against the parasitic leeches.

CONCLUSION

The results showed that the methanol extract of *D. suffruticosa* flower significantly kill all the parasitic marine leeches *Z. arugamensis* at various concentrations 25, 50 and 100 mg/ml, at an average period of 53.77 ± 1.42, 37.63 ± 5.35 and 7.51 ± 0.74 min, respectively compared to the normal control group. Thus, it is indicated that the flower of the plant can act as an effective antiparasitic agent in aquaculture. This is the first study on studying the effects of flowers against parasites and this novel approach will certainly be beneficial to fish farmers to control the parasites in aquaculture in Sabah, Malaysia.

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