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EFFICACY OF TURMERIC CURCUMA LONGA RHIZOME EXTRACT AGAINST THE MARINE PARASITIC LEECH IN AQUACULTURE

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Abstract: *Zeylanicobdella arugamensis* (Annelida, Hirudinea, Piscicolidae) is a marine parasitic leech that poses a serious threat to the aquaculture industry in Malaysia, particularly in cultivated hybrid groupers. The turmeric plant, a rhizome of *Curcuma longa* (Spermatophyta, Zingiberaceae), locally known as "Kunyit," has been utilised in this study to elucidate its antiparasitic efficacy as a natural biological control method against *Z. arugamensis*. The parasitic leeches were hand-picked from a heavily infested hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*). These parasitic leeches were evaluated against a methanol extract of *C. longa* rhizome. The study was conducted *in vitro,* and the extracts were applied at 25, 50, and 100 mg/mL doses. Notably, at a dosage of 100 mg/mL, significant parasitic leech mortality was observed within a short period of 6.12 minutes. Before their mortality, we keenly observed their behavioural changes, such as altered motility and the inability to connect to the surface via suckers. Thus, our study indicates that the extract of *C. longa* rhizome demonstrated significant antiparasitic activity against *Z. arugamensis*.

Keywords: Aquaculture, turmeric, rhizome, curcuma, leech, *Zeylanicobdella arugamensis*, natural product, hybrid grouper, biological control.

Introduction

An ectoparasitic leech *Zeylanicobdella arugamensis* (Annelida, Hirudinea, Piscicolidae) affects a diverse range of fish species and poses a serious threat to the aquaculture industry. However, because of global environmental changes, the ectoparasitic leech was found in several host fishes along the coasts of several countries, which include Malaysia, Thailand, Indonesia, Brunei Darussalam, Singapore, India, Iran, the Philippines, Australia, and Japan (Nagasawa & Uyeno, 2009; Venmathi Maran *et al*., 2009; Venmathi Maran *et al*., 2012a, 2012b; Ravi & Yahaya, 2017; Azmey *et al*., 2020). Apart from parasitic caligiform copepods (Boxshall *et al*., 2008; Venmathi Maran *et al*., 2009), the presence of marine leeches in a cage-rearing Asian sea bass, *Lates calcarifer*, has been documented (Kua *et al*., 2006). It was found that several juvenile leeches hatch the most at a salinity of 30 ppt, followed by 20 ppt and 10 ppt. The proportion of Asian sea bass fry infected with these parasitic leeches can reach up to 70% after two weeks in the cages, causing scale disintegration, tail, and fin infection. At salinities ranging from 10 to 40 ppt and temperatures ranging from 25 to 35°C, adult and juvenile leeches were found to live for an average of four to seven days (Kua *et al*., 2006). Parasitic leech infestations result in the mortality of the host, often within three days following a secondary infection by the bacteria *Vibrio alginolyticus*. The presence of secondary infection can exacerbate the fish's health and lead to death (Gopalakrishnan *et al*., 2011a; Gopalakrishnan *et al*., 2011b; Kua *et al*., 2014; Suyanti *et al*., 2021).

Therefore, *Z. arugamensis* is a significant danger to the aquaculture industry, and prevention methods are essential. Due to the lack of biocontrol agents, toxic chemicals have been used by farmers, including formalin, copper sulphate, organophosphate, sulfonamides etc., for parasitic disinfestation (Shariff *et al*., 2000). The toxic chemicals are harmful to fish

as well as humans and the environment. These toxic chemicals cause genotoxicity, enzyme inhibition, gill damage, mucous cell alterations, haematological changes, hypochloremia, skin ulceration, and mortality in fish (Narra *et al*., 2012; Leal *et al*., 2018; Resendes *et al*., 2018; Shah *et al*., 2020a). In human beings, these chemicals induce cancer, nephrotoxicity, and nervous system damage (Marrs, 1993; Shah & Iqbal, 2010; Yang *et al*., 2020). While the release of these harmful chemicals into the environment, negatively affects water quality (declines dissolved oxygen, pH, etc.), induces biological toxicity, and causes accumulation (Rowland *et al*., 2006; Liu *et al*., 2021).

Medicinal plants are of great importance because of their unique properties, such as having a rich source of therapeutic phytochemicals that could lead to the creation of new medications (Wink, 2012). Most phytochemicals generated from plants that contain phenolics, alkaloids, flavonoids, and other compounds have been found to have a favourable effect and can be a good option to treat parasitic infections and consequent diseases (Muhammad *et al*., 2003; Bahmani & Rafieian-Kopaei, 2014; Othman *et al*., 2019; Shah *et al*., 2020a).

Curcuma longa Linn. (Turmeric), locally known as *Kunyit*, is a rhizomatous, herbaceous perennial plant in the Zingiberaceae family. Its extract has been shown to have a variety of properties, including hepatic and cardioprotective (Mohanty *et al*., 2004; Rivera-Espinoza & Muriel, 2009) hypoglycaemic (Honda *et al*., 2006), anti-amyloidogenic (Shytle *et al*., 2009), antifungal (Cho *et al*., 2006), antioxidant (Cousins *et al*., 2007), antibacterial (Niamsa & Sittiwet, 2009) and anticancer (Kuttan *et al*., 1985). *C. longa* extract has also been reported to have parasuicidal activity against *Plasmodium, Leishmania, Trypanosoma,* nematodes, *Giardia,* etc. (Haddad *et al*., 2011). Since there is no research on *C. longa*'s antiparasitic activity against this particular parasitic leech *Z. arugamensis*, it is considered a novel work. The major aim of this study is to test the antiparasitic activity of *C. longa* methanol extract against *Z. arugamensis* at different concentrations/dosages.

Materials and Methods

Sample Collection

The rhizome of *C. longa* was obtained from the local market at Kota Kinabalu (5.9804° N, 116.0735° E), Sabah, Malaysia, and rinsed with distilled water. After that, it was cut into thin layers and placed in an oven at 37°C for four days. The dried turmeric roots were finely pulverised and stored in an airtight plastic jar.

Sample Extraction and Preparation

Around 145 g of dried turmeric powder was dissolved in 250 mL of HPLC-grade methanol and agitated at room temperature using an orbital shaker (Model: IBS-NR-25-8) for 72 hours. Whatman No. 41 filter paper was used to filter the supernatant. The methanol residues were completely removed from the extract using a vacuum rotary evaporator until it became saturated. After being stored in a -80°C freezer for 24 hours, the saturated crude extracts were then freeze-dried using a freeze dryer, yielding approximately 18 g of dried extract (Shah *et al*., 2021).

Further, for the experiment, three extract concentrations (25, 50 and 100 mg/ml) were selected. To prepare these concentrations, the dried methanol extract was dissolved in seawater containing 2% Tween 80. The mixture was then sonicated for 5 minutes to enhance the solubility, followed by filtration using a syringe filter to remove undissolved particles. The resulting solution was used for further research and analysis.

Parasitic Leech Collection

The parasitic leeches, *Z. arugamensis* (1-1.5 cm), were obtained from the aquaculture facilities of Universiti Malaysia Sabah. The parasitic leech-infested hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) (weight: 250-350 g, diameter: 15-25 cm) caught from the cage was transferred into a small tank filled with seawater, and the leeches were manually removed (Figure 1). The collected leeches were transferred into another container

filled with filtered seawater and incubated for 24 hours at 27°C before the experiment was conducted.

Antiparasitic Bioassay

A total of 60 mature and healthy parasitic leeches were used. The leeches were divided into five groups (4 leeches/group), Group 1 served as a negative control and was treated with 1 mL of seawater. Group 2, on the other hand, served as a positive control and received 1 mL of a 0.25 % v/v formalin solution.

For Groups 3, 4, and 5, the parasitic leeches were exposed to 1 mL of the methanol extract of *C. longa* at different concentrations of 25, 50, and 100 mg/mL, respectively. The treated parasites were placed in a glass petri dish and observed more than 1 hour (Shah *et al*., 2021). The experiment was done in triplicate to assure the accuracy and reliability of the results.

The Behaviour of Parasitic Leech and Mortality Time

Throughout the experiment, the behaviour of the parasitic leeches in both the control and extract-treated groups was visually observed. This includes monitoring swimming patterns, body movement and attachment to the solid surface using their anterior and posterior suckers.

Furthermore, the mortality time of the parasitic leeches exposed to the extract was recorded using a stopwatch. To determine the point of mortality, parasitic leeches were considered dead when they displayed no movement even after being physically prodded with a needle on multiple occasions (Venmathi Maran *et al*., 2021).

Physico-Chemical Parameters

Water quality parameters such as pH, dissolved oxygen, temperature, and salinity were recorded in the control and treated groups using a multi-item water quality meter, Hyogo, Japan.

Statistical Analysis

The data was analysed using the IBM SPSS Statistics 25 Window application (IBM, Armonk, NY, USA). To find significant differences between groups, one-way analysis of variance (ANOVA) was utilised, followed by Tukey's multiple comparisons test. All of the results were presented using the mean and standard error of the mean (S.E.). *P* values of less than 0.05 were deemed significant.

Results

Mortality Time

The fatality time of the parasitic leeches tested with a methanol extract of *C. longa* is shown in Figure 2. Parasitic leeches treated with seawater (negative control) indicated no mortality in > 60 minutes. Parasitic leeches exposed to formalin

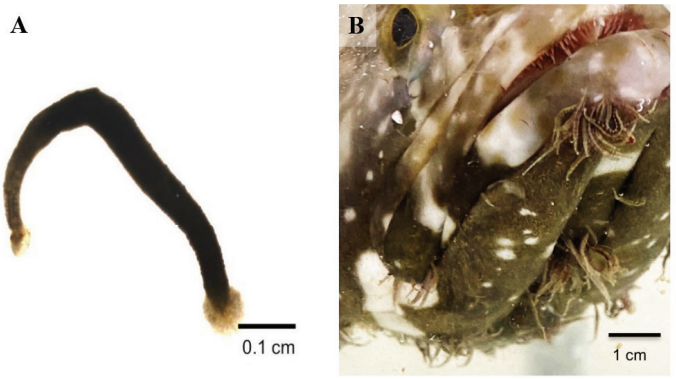

Figure 1: A = Adult *Zeylanicobdella arugamensis,* B = Arrows showed the infestation of *Zeylanicobdella arugamensis* on the upper and lower jaw of the hybrid grouper

indicated complete mortality in 2.20 ± 0.02 minutes. Similarly, parasitic leeches exposed to methanol extract showed complete mortality at all concentrations. However, parasitic leeches treated with methanol extract at a concentration of 100 mg/mL showed total mortality in a shorter time of 6.12 ± 0.03 minutes as compared to 50 mg/mL (8.06 ± 0.06 minutes) and 25 mg/mL (10.50 ± 0.04 minutes) with significant differences.

Mortality Percentage

Table 1 shows the mortality percentage of parasitic leeches administered with varying concentrations of *C. longa* methanol extract. The control group was treated with seawater and indicated no mortality, but the formalin and extract-treated groups showed complete mortality.

Physico-chemical Parameters

All parameters determined were consistent, except for a change in pH values between the control and the plant treatment groups. Table 2 indicates the water quality parameters in the control and treatment groups during the experiment. The pH values of the negative control and positive control groups (7.86 and 7.24) indicated variations in pH values compared to the *C. longa* methanol extract groups at a

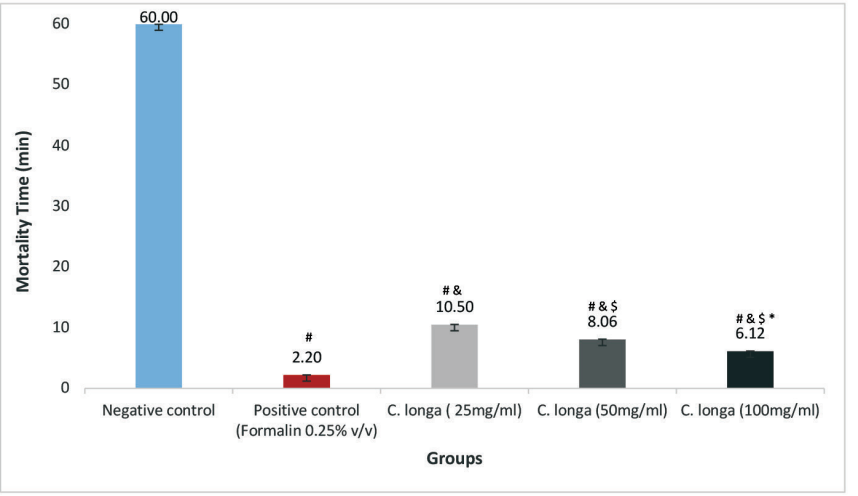

Figure 2: Mortality time of parasitic leeches treated with different concentrations of ethanol extract of *C. longa*, negative and positive control groups

Note: Each value represents the mean ± S.D of four leeches per group.
[#]Significance at p = 0.05 compared with the normal control group.
[&]Significance at p = 0.05 compared with the formalin (0.25% v/v).
[$]Significance at p = 0.05 compared with *C. longa* (Methanol 25 mg/mL).
[*]Significance at p = 0.05 compared with C. longa (Methanol 50 mg/mL).

Table 1: Mortality percentage of parasitic leeches at different concentrations of methanol extract of *C. longa*

No.	Groups	Mortality (%)
1	Negative control (Seawater)	0
2	Positive control (formalin 0.25%) (v/v)	100
3	*C. longa* (25 mg/mL)	100
4	*C. longa* (50 mg/mL)	100
5	*C. longa* (100 mg/mL)	100

Table 2: Water quality parameters of control and treatment groups in methanol extraction

Water Parameters	Concentrations				
Groups	Control	Formalin	*Curcuma longa*		
		0.25%	(100 mg/mL)	(50 mg/mL)	(25 mg/mL)
Temperature (°C)	24.0	24.0	24.0	24.3	24.1
pH	7.86	7.24	4.05	5.42	5.91
Dissolved Oxygen (mg/L)	5.0	6.30	6.32	5.21	4.95
Salinity (ppt)	37.0	37.0	37.0	37.0	37.0

concentration of 100 to 25 mg/mL (4.05 to 5.91), respectively. The pH fluctuations could be caused by the presence of acidic bioactive chemicals in the *C. longa* extract-treated groups.

The Behaviour of Z. arugamensis

The locomotive behaviour of parasitic leeches treated with seawater remained unchanged throughout the observation period. However, in the formalin-treated group, significant discomfort and stress were noticed in the first minute. The parasitic leeches were swimming sideways, and their movement was not organised.

Similarly, the behaviour of parasitic leeches treated with the methanol extract of *C. longa* showed similar results. With no organised movement, they were unable to cling to the bottom of the glass petri dish using their suckers, indicating a disruption in their behaviour. Notably, the group treated with the highest concentration of 100 mg/mL displayed the highest level of stress among the leeches, followed by the groups treated with concentrations of 50 mg/mL and 25 mg/mL.

Discussion

The marine parasitic leech *Z. arugamensis* is proving to be a serious menace to the aquaculture industry, especially the grouper culture industry in Southeast Asian countries (Cruz-Lacierda *et al*., 2000; Ravi & Yahaya, 2017). Chronic anaemia is common in heavily infected fish with leeches (Noga, 2000). When a grouper's skin is infested with leeches, it will brush its body against objects in its environment, creating injuries and ulcers on the skin or in the mouth (Noga, 2000; Shah *et al*., 2020a). Furthermore, the ectoparasitic leech serves as a vector for disease transmission, causing the host fish to die within a short time (Burreson, 2006; Kua *et al*., 2014). To control and eliminate the parasitic infestation, various toxic chemicals have been applied by farmers. The chemical method employed by fish farmers has a negative impact on fish, humans, and the environment (Mohamed *et al*., 2000; Nagasawa & Cruz-Lacierda, 2004). Thus, for the control of parasitic infestations, aquaculture should focus on solutions that are both ecologically friendly and long-lasting. Plant-based disease management in aquaculture has recently gotten a lot of attention as a viable alternative to chemical treatments (Reverter *et al*., 2014). The antiparasitic potential of the methanol extract of *C. longa* was investigated in the present study. At a concentration of 100 mg/mL, the extract killed parasitic leeches in approximately 6 min, followed by 8 and 11 min at 50 mg/mL and 25 mg/mL, respectively.

In a similar study, the methanol extract of the medicinal plant *Dillenia suffruticosa* (Dilleneacea), locally known as *Simpoh air* or *Simpoh ayer*, was tested against Z. *arugamensis*. The parasitic leeches were subjected to the methanol extract of *D. suffruticosa* solution, which killed all the leeches in 14.39 minutes. at the concentration of 100 mg/mL, followed by 50 mg/mL and 25 mg/mL in 32.97 and 41.77

minutes, respectively (Shah *et al*., 2020b). Following that, another medicinal plant, *Nephrolepis biserrata* (Nephrolepidaceae), locally known as *Paku larat* was tested with methanol extracts and 100% mortality of leeches was found. The average time recorded to kill leeches at 100, 50, and 25 mg/mL concentrations was 4.88, 11.91, and 25.11 minutes, respectively. Additionally, hybrid groupers were also disinfested with exposure to the methanol extract of the plant which resulted in the complete disinfestation of the host fish in less than 30 min. at a concentration of 10 mg/mL (Shah *et al*., 2020a). The methanolic extracts of garlic *Allium sativum* L. (Amaryllidaceae) have been reported with significant antileech activity at a concentration of 600 µ/mL against the parasitic leech *Limnatis Nilotic* in a time limit of 68 minutes. Similarly, the antileech activity of *Zingiber officinale* (Zingiberaceae) against *L. nilotica* has also been reported. The methanol extract of the plant at a concentration of 32×10^4 ppm resulted in the mortality of *L. nilotica* in 24 minutes (Forouzan *et al*., 2012).

The bioactive chemical constituents of turmeric have been investigated based on a previous study (Li *et al*., 2011; Venmathi Maran *et al.,* 2022; Shah *et al*., 2022). *Curcuma longa* contains approximately 235 compounds, the majority consisting of phenolics and terpenoids, such as diarylheptanoids (commonly referred to as curcuminoids), diarylheptanoids, monoterpenes, sesquiterpenes, diterpenes, triterpenoids, alkaloid, and sterols. The major bioactive constituents are curcuminoids, primarily curcumin, and essential oils, primarily monoterpenes, which have been reported to have antiparasitic activity against animal parasites (Li, 2011; Cervantes-Valencia *et al*., 2019). Hence, it can be concluded that the antiparasitic activity of *C. longa* methanol extract is attributable to the presence of antiparasitic bioactive chemicals, particularly curcuminoids.

Conclusion

The results of the current experiment distinctly showed that the methanol extract of the turmeric *Curcuma longa* rhizome possessed high antiparasitic activity against the parasitic leech *Zeylanicobdella arugamensis*. In this study, the methanol extract of *C. longa* with concentrations of 100, 50, and 25 mg/mL was applied, which resulted in 100% mortality of parasitic leeches in periods of 6.12, 8.06, and 10.50 minutes, respectively. This result suggests that the higher the methanol extract concentration, the shorter the fatality time of the parasitic leech. This is the first study conducted on the antiparasitic effect of *C. longa* against the parasitic leech. Thus, our study indicated that the extracts of *C. longa* rhizome demonstrated significant antiparasitic activity against the parasitic leeches, particularly at 100 mg/ml concentration due to less mortality time. However, further research is needed to identify and isolate the pure bioactive compounds responsible for the antiparasitic potential.

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